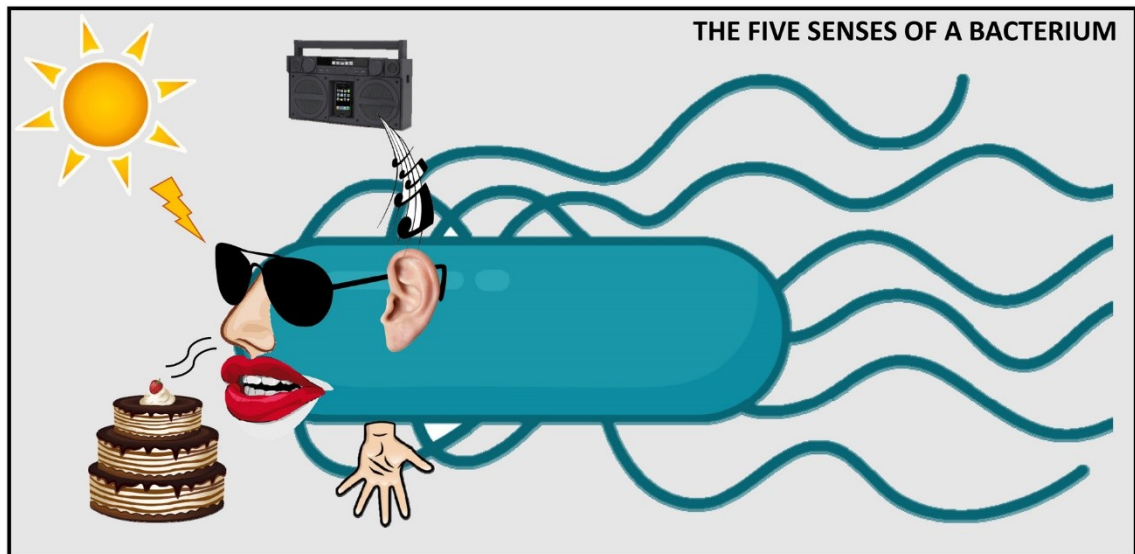


Sensing the environment

*Miss: Can bacteria see, hear, smell, taste and touch?
Can they talk to their friends?*



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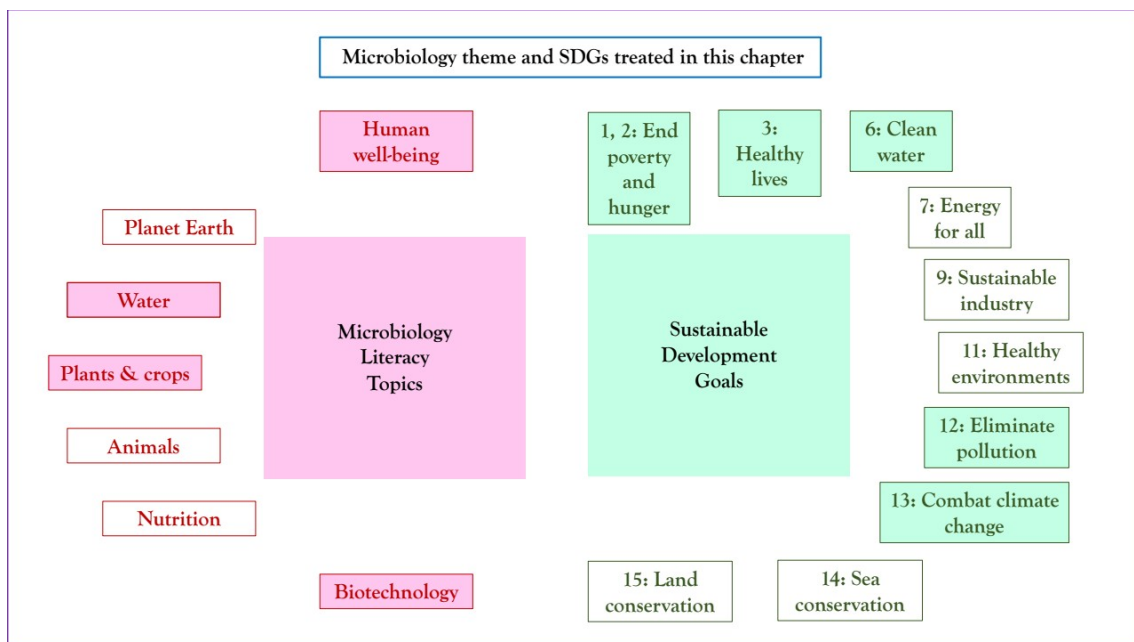
Sensing the environment

Storyline

We humans perceive our environment with the help of 5 senses, namely sight, hearing, touch, smell and taste. These senses permit us to monitor the environment. Who has not enjoyed smelling and eating their favourite food, or attending a concert of their preferred music band? Who has not experienced the pain of a grazed knee after a fall or enjoyed a sunset on the beach? Through these 5 senses we collect crucial information that in turn forms the basis of decision taking. But what is true for humans might also be true for bacteria. In fact, humans are half bacteria and half human. In other words, a healthy person has approximately the same number of bacterial cells in his or her body as human cells. However, as unicellular entities, bacteria are much more exposed to changes in the environment, which reinforces the need to sense their surroundings. This external information is gathered by bacteria through a complex system of monitoring devices, or receptors, that allow these microscopic unicellular entities, for example, to detect light, to know where there is food, or where they can comfortably establish themselves. In analogy to humans, bacteria use this information to take fundamental decisions. The ability to interfere with this information gathering process is a means to fight bacteria that are harmful to humans and, at the same time, permits a more optimal exploitation of the capacities of beneficial bacteria.

The Microbiology and Societal Context

The microbiology: microbial sensing of environmental signals; bacterial receptors; bacterial metabolism; chemotaxis; antimicrobial resistance; biofilm formation; plant-bacteria symbiosis; microbial cell factories. *Sustainability issues:* global food supply; plant, animal and human health; clean water; environmental pollution; climate change.



Sensing the environment: the Microbiology

1. ***Bacteria have multiple strategies to sense environmental signals.*** In our anthropocentric view of the world, we consider microorganisms as simple entities. Nothing could be further removed from the truth than this. Thousands of research studies have demonstrated that bacteria employ highly sophisticated mechanisms that allow them to control their movements, metabolism, growth or the transition to dormancy (e.g. spore production). The coordinated action of these activities is achieved by monitoring different external and internal stimuli through multiple sensors (= sensor proteins), that are present inside and on the surface of the microbial cell. In the language of our contemporary technology-based era, this would correspond to thousands of highly sensitive microsensors capable of detecting an enormous diversity of physical and chemical stimuli. Given this high sensing capability, could we say that bacteria have five senses? To a certain extent they have, as illustrated below.

2. ***The ability of bacteria to detect nutrients: taste.*** Organisms need food to survive, grow and multiply. However, not all organisms are adapted to consume different types of food. For example, mammals can be classified according to their diet type into carnivores, omnivores and herbivores, and they have different physiological and anatomical features that are directly associated with each of these diet types. Bacteria also need food, mainly nitrogen (e.g. amino acids, polyamines) and carbon (e.g. sugars, organic acids), to grow and multiply. Nevertheless, the ranges of food a given bacterium uses can be very narrow since they may lack the ability to metabolize certain nutrients that other bacteria can use.

Likewise, in nutrient-rich environments, bacteria preferentially “consume” certain compounds over others. This is comparable to choosing your favourite dishes from a buffet in a restaurant. Thus, in conditions where there is a mixture of different foods, bacteria can selectively metabolize one particular compound (i.e. a nutrient that allows a faster growth) and, at the same time, restrict the consumption of less favourable nutrients. This process is known as carbon catabolite repression, and is a complex regulatory mechanism that implies the sensing of particular carbon sources by bacterial sensor proteins, ultimately resulting in the activation of the metabolic pathways of preferred carbon sources and the repression of those pathways required for degradation of non-preferred compounds. This food preference can be compared to human taste.

Additionally, sensing of preferred foods by bacterial receptors can also modulate additional behaviours such as the movement towards nutrients. It has been estimated that approximately half of the bacteria are able to move rapidly towards favourable compounds like foods, or away from undesirable, frequently toxic compounds, by the process known as chemotaxis. This mechanism also depends on the bacterial flagellum, a whip-like appendage that allows bacteria to swim at high speeds through water or water films on surfaces. This strategy is critical for bacterial survival in their natural habitats as it confers the possibility of accessing areas that are more suitable for growth or with higher nutrient concentrations.

3. ***“Bacterial noses” or how bacteria detect volatile compounds: smell.*** Animals, plants and microbes produce and secrete a vast array of organic and inorganic volatile compounds – compounds that readily evaporate and diffuse into the air – that can be sensed by physically separated (micro)organisms and thereby act as signal molecules, permitting chemical conversations between different species. Volatile compound-mediated communication in bacteria is a new research area and the mechanisms by which bacteria sense and respond to these chemicals remain largely unknown. However, the detection of these volatile compounds

by bacteria has been shown to control a broad range of processes, including virulence, antibiotic synthesis, growth, movement and **biofilm** formation.

Not surprisingly, volatiles play important roles in the interactions between bacteria and plants. For example, using specially designed “olfactometer” devices, it was shown that bacteria can detect odors (e.g. volatile compounds) that are produced by their plant hosts causing the attraction of soil bacteria to plant roots. Another example is the “cry for help” of plants that become infected by fungi, through the production of volatile compounds that are the “smelled” by bacteria which respond by producing compounds that help plants to overcome the infection. Sensing the smell of plants permits many bacteria to move towards the plant permitting the use of food compounds released by the plants. In return, bacteria help the plant in its fight against pathogens.

4. *Surface sensing by bacteria: touch.* Bacteria can attach to biotic (e.g. human tissues, plant roots) and abiotic (e.g. surgical instruments, catheters) surfaces to form complex and dynamic bacterial communities known as biofilms. Living in a biofilm confers multiple advantages to bacteria, including resistance to drugs (e.g. antibiotics, toxins) and environmental stresses (e.g. ultraviolet light, dehydration), a greater access to nutrients, and an increased gene transfer within the biofilm. Biofilm formation starts with the initial contact between a bacterium and a surface. Consequently, bacteria must know that they are on a surface in order to trigger a series of processes that will initiate the biofilm formation process. This mainly occurs because bacteria can sense surface contact that may be the equivalent to the human sense of touch. Surface sensing is a complex multi-step process in which different bacterial surface appendages such as the above-mentioned flagellum or membrane-associated receptors act as surface sensors. Once the surface has been detected and explored, bacteria activate a process that triggers a cascade of internal signaling events leading eventually to biofilm formation.

5. *Light detection in bacteria: sight.* Sight is a magic sense. Through our eyes, we receive different light stimuli that are eventually converted into electrical signals and sent through the optic nerve to the brain for interpretation. Some bacteria can also sense different wavelengths of light and respond accordingly to the light stimulus they receive. This process occurs due to the action of light-sensing proteins, the so-called **photoreceptors**. The best characterized bacterial photoreceptors are those of the cyanobacteria, photosynthetic bacteria that are present in diverse ecosystems, from oceans to deserts. Given that cyanobacteria depend on sunlight for growth and survival, they have dozens of different photoreceptors, some of which can sense a wide range of light wavelengths, from infrared to ultraviolet. Through these light-sensing proteins, cyanobacteria are able to modulate crucial cellular processes, including bacterial growth, phototaxis (i.e. movement towards or away from a light source), biofilm formation and their photosynthetic activity. However, photoreceptors are not restricted to photosynthetic bacteria and are also frequently present in plant-associated bacteria, which represents an astonishing parallelism with their photosynthetic hosts. This parallelism is not a mere coincidence. Light regulates for example seed germination or plant defence against pathogens. Furthermore, many plant-associated bacteria have the ability to coordinate specific behaviours with the physiology of the host plant by sensing light. Such plant-bacteria coordination is especially relevant for establishing **symbiosis**, or in other words, mutually beneficial plant-bacteria interactions. However, light sensing is also important for pathogenic bacteria to modulate, for example, nutrient metabolism, motility, biofilm formation or virulence.

6. **Can bacteria respond to sound?: hearing.** No one likes to be shouted at. Things should be said in the right tone and volume. The same seems to be true for bacteria. High-power ultrasound is a physical method used to efficiently kill bacteria and bacterial spores. In fact, ultrasonic cleaning is currently employed, for example, in the sterilization of medical and surgical devices and equipment. In addition, low intensity ultrasound is a safe and non-invasive strategy for the enhancement of the effect of antibiotics on bacteria. Alternatively, there is also evidence for the capacity of bacteria to detect and respond to sound waves in the audio frequency range. It was found that low-frequency ultrasound treatment enhances bacterial growth and stimulates the germination of bacterial spores. These processes are currently poorly investigated and further research will determine the mechanisms behind these sound-controlled bacterial activities.

7. **Interfering with bacterial sensing as an alternative to antibiotics.** Currently, our major weapons to fight bacteria that cause infections are antibiotics that either kill or slow down the growth of bacteria. However, the excessive use of antibiotics increases the appearance of antibiotic-resistant bacteria, an issue that is one of the most serious current global health challenges. In fact, antimicrobial resistant bacteria kill approximately 700 000 patients per year, and estimations indicate that the number of deaths due to resistant bacteria will rise to 10 million per year by 2050, surpassing the number of deaths due to cancer. Therefore, we need new strategies to combat antibiotic resistant bacteria. Interfering with bacterial sensing, i.e. blocking or altering one or more of the five senses of bacteria, is a promising alternative approach. This strategy will neither kill nor slow down bacterial growth and, as a consequence, will not select for the rapid emergence of resistant bacteria. Specific chemicals can be used to make the bacterium perceive the environment wrongly, for example, to pretend that there are few bacteria when there are many, that a certain type of food is unavailable whereas it is available or inhibiting bacterial motility. Based on this wrong information, bacteria will take erroneous decisions that can either be fatal or represent a large handicap that inhibits bacterial fitness and virulence.

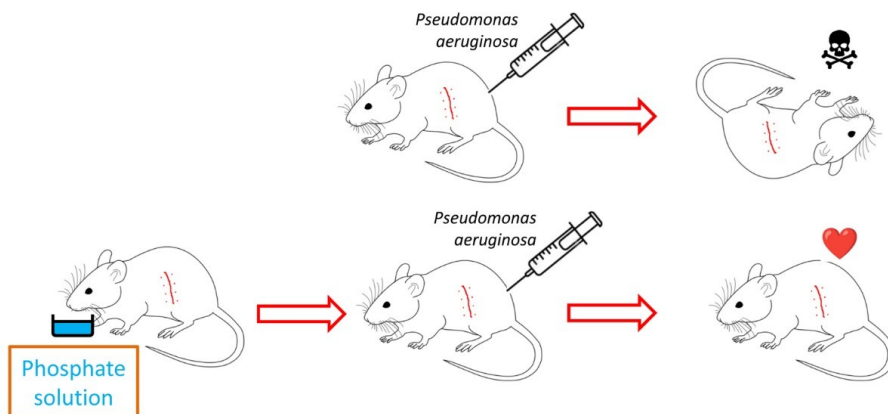
Box 2: The application of the natural molecule phosphate to mice prevents death by *Pseudomonas aeruginosa* infection.

Scientific data:

- Hospital-acquired infections with the bacterium *Pseudomonas aeruginosa* are the cause of death and suffering.
- Low concentrations of phosphate are sensed by *P. aeruginosa* and increase the virulence of the bacterium.
- Major surgery reduces human phosphate concentrations favouring infections by *P. aeruginosa*.

Development of an antimicrobial strategy:

- Oral administration of a phosphate solution to mice prevents death by *P. aeruginosa* infection.



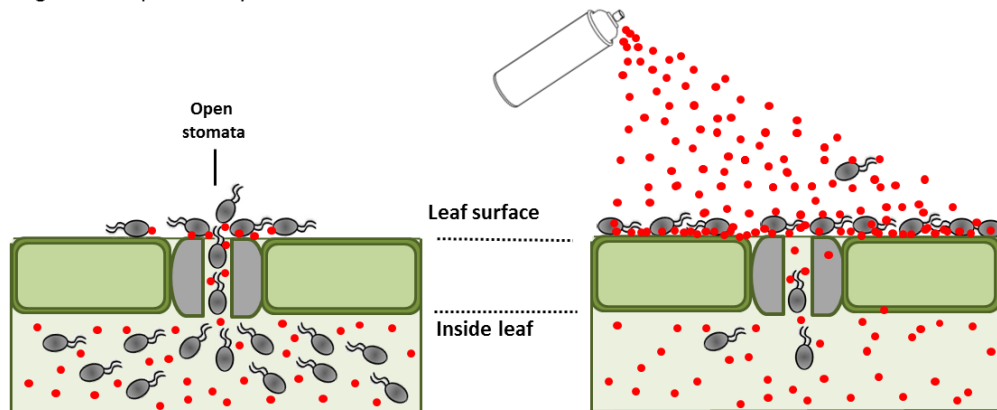
Box 3: Perturbing compound gradients on the plant leaf surface disorients bacteria leading to a reduced plant entrance and reduced severity of infection.

Scientific data:

- Many disease causing bacteria enter the plant through openings, i.e. wounds or stomata.
- Bacteria are attracted to stomata through directed movements towards compounds released by the stomata (red dots), this attraction facilitates bacterial entry and favours infection.
- Key compounds have been identified that are critical for this chemoattraction.

Development of an antimicrobial strategy:

- The application of these key compounds to the leaf surface disorients the bacterium causing reduced plant entry and less severe disease.



8. *There is sensing prior to symbiosis.* In general, society associates bacteria with disease and uncleanness, but less widespread is the notion that many bacteria are beneficial to human and animals (i.e. aid digestion and nutrient absorption) or plants (i.e. protection against diseases). However, terms such as **microbiota**, defined as a complex assembly of microbes present in different sites of a host (e.g. gut, plant roots, plant leaves, etc.) is becoming increasingly familiar to society. The beneficial effect of bacteria to human, animal and plant health is to a large degree due to their capacity to participate in symbioses - a beneficial interaction between different organisms that live in close physical association. One typical example of symbiosis is the mutualism between the gut microbiota and the host, where the microbiota plays an important role in food digestion in the host while, in return, the microorganisms obtain nutrients and a privileged habitat. Prior to the establishment of a symbiotic interaction, the interacting partners need to find each other, a process that is typically based on sensing signals released by the other organism. For example, during the symbiotic interaction between soil bacteria and leguminous plants (plants that form root nodules containing bacteria that convert nitrogen gas in the atmosphere to a form that plants can use), the former sense different compounds released from the plant roots (e.g. amino acids, organic acids, sugars, flavonoids) and are thereby attracted towards the host roots where they create the development of root **nodules**. Symbiotic interactions between plants and bacteria are highly relevant for agricultural applications since they can promote plant growth (i.e. plant yield) and render the plant more resistant to attack by pathogens. Knowledge about how bacteria sense plants permits us to engineer bacteria that are more efficient in their capacity to find the “partner” and establish symbiosis.

9. *Understanding sensing mechanisms is essential for the biotechnological exploitation of microbes.* In industrial microbiology, microorganisms are used as microbial cell factories to produce a number of value-added compounds such as biofuels, antibiotics, polymers, enzymes, amino acids or organic acids. As a consequence, such microbes form the basis for biotechnological **biotransformation** processes that correspond to a bacteria-based mode of synthesizing a given chemical. Frequently, the production yield of such green chemistry approaches is superior to that of synthetic chemistry alternatives. The importance of biotransformation is illustrated by the fact that there are currently more than 21,000 patents that contain the term “biotransformation” in the title or abstract. To improve the yield of the desired value-added products, microbes can be manipulated in a way that they produce in excess a given compound. Metabolic activities within a bacterial cell are frequently tightly regulated by sensor proteins, which restrict the yields of desired compounds. Understanding sensing mechanisms is therefore essential to develop high yielding, efficient green chemistry biotransformation applications.

Many bacterial sensor proteins are highly specific for a given chemical compound and such proteins can be used to construct sensor devices (**biosensors**) that we can use to detect compounds of interest. Such biosensors recognize a target compound (i.e. a pollutant present in contaminated soils or waters) and generate in return an easily visible signal, like a colour change, which is indicative of pollution.

Relevance for Sustainable Development Goals and Grand Challenges

- **GOALS 1 and 2: End Poverty and Hunger.** The world's population keeps growing and is expected to reach 10 billion by 2050. In order to feed this increasing population, current estimations indicate that agricultural production will require an increase of approximately 70% over current levels. To achieve this increase, the use of plant beneficial bacteria is an ecologically-friendly approach. There are many examples showing that symbiosis of bacteria with agricultural crops promote their growth and consequently yield. In addition, other examples show that bacteria-plant symbioses result in an increased resistance of the plant towards a pathogenic attack by other microbes. By exploiting knowledge on sensing, bacteria can be modified in a way that they engage faster and in a more beneficial type of symbiosis.
- **GOAL 3: Healthy lives.** The increasing emergence of pathogenic bacteria that are resistant to all available antibiotics, known as multidrug resistant bacteria or superbugs, is currently a major global health threat. In addition to the development of novel antibiotics, fighting pathogenic bacteria with means other than antibiotics or pesticides will reduce the possibility of generating antibiotic-resistant bacteria and will have a lesser impact on the environment, as well as on human and animal health. Many bacterial sensor proteins control aspects that are directly related to the capacity of some bacteria to cause disease. There are a number of examples where the interference with different bacterial sensor mechanisms has resulted in an attenuation of bacterial virulence (see Box 2 and 3). The proof of this concept has been obtained for bacteria that infect human as well as plants, but further efforts are required to transfer the obtained knowledge into products.
- **GOAL 6: Clean Water.** At present, antibiotics and pesticides are our main weapons to fight pathogenic bacteria and the use of chemical fertilizers represents currently the main strategy to promote plant growth. However, the excessive (and many times unjustified) use of these chemicals has a detrimental impact on the environment and, in particular, on the water

A child-centric microbiology education framework

quality. Thus, chemical fertilizers and pesticides leach into the groundwater and end up in rivers, lakes or oceans. Additionally, antibiotics present in urban waters can persist after their treatment in wastewater plants that are then released into the environment. Consequently, alternative strategies based on interfering with the sensing of bacterial pathogens as well as on the development of more efficient plant-growth promoting bacteria will reduce the amount of antibiotics, pesticides and fertilizers released into the environment. Furthermore, the development of bacterial biosensors with a determined signal specificity can be used to construct analytical tools for a rapid monitoring of the water quality.

- **GOAL 12: Responsible Consumption and Production.** Similar to Goals 1 and 2, the importance of the plant-associated bacteria for plant growth and health reinforces their use as **biofertilizers** and **biopesticides** in agriculture. In fact, the use of microbially-based products to improve agronomic yields and to reduce production costs in an environmentally-friendly manner is increasing significantly worldwide. Deciphering the mechanisms by which beneficial plant-associated bacteria sense environmental signals during their interaction with host plants will lay the groundwork for improving the performance of future microbial-based agricultural technologies.

- **GOAL 13: Combat climate change.** Global warming, fossil fuel depletion and the increase in global energy demand is prompting interest in the use of microorganisms for the conversion of starting materials (e.g. food crops, woody biomass) into biofuels (e.g. bioethanol, biodiesel, biogas). For an efficient conversion, the microorganisms must be able to tolerate high concentrations of the final biofuel, but also possess a high metabolic activity for substrate conversion. Consequently, the identification of signal molecules that activate bacterial metabolism would potentially increase the yield and productivity of biofuel-producing bacteria.

Potential Implications for Decisions

1. *Individual*

- a. Avoid taking antibiotics unnecessarily.
- b. Avoid wasting food.
- c. Do not aim having a sterile, bacteria-free home as suggested by publicity.
- d. Reduce the use of chemical pesticides, not only in large-scale agriculture but also in small farming.

2. *National Policies*

- a. Promote research on the inhibition of pathogenic bacteria by non-antibiotic approaches.
- b. Reconsider the policies for the use of genetically modified bacteria in agriculture.
- c. Promote the use of microorganism-based technologies for the development of green and sustainable cities.

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Pupil participation

1. Class Discussion

- a. What do you think is the sense that is most important to humans, and what is the sense that is most important to bacteria?
- b. Did you know that many bacteria can distinguish between different types of food (taste) and that many bacteria have a favourite food?
- c. For many bacteria sugars are the preferred food. Do you see any parallels with you?
- d. Did you know that many bacteria can swim towards places where there is their favourite food?
- e. Bacteria reach speeds that correspond to 30 times their size in a single second. In proportion to your size, are you able to run that fast to a bar of chocolate?

2. Exercises

- a. Look at different places and objects in your home and try to locate biofilms. Where do you think would be the most common places for bacteria to establish themselves in your home?
- b. Look up in the internet the name of a sugar that is secreted by plant roots and that attracts bacteria.
- c. Navigate in the Internet to <https://peerj.com/articles/3107/#>. In Figure 3 compare the size of tomato plants in the absence and presence of the bacterium RWL-1 (left) and tomato plants infected with the pathogenic fungus *F. oxysporum* in the absence and presence of the bacterium RWL-1 (right). Interpret the results of this image.

The Evidence Base, Further Reading and Teaching Aids

More than half your body is not human. <https://www.bbc.com/news/health-43674270>
Revised Estimates for the Number of Human and Bacteria Cells in the Body. <https://journals.plos.org/plosbiology/article?id=10.1371/journal.pbio.1002533>
Tackling drug-resistant infections globally: final report and recommendations. http://amr-review.org/sites/default/files/160518_Final%20paper_with%20cover.pdf
Microbial volatile organic compounds in intra-kingdom and inter-kingdom interactions. <https://www.nature.com/articles/s41579-020-00508-1>
Calling from distance: attraction of soil bacteria by plant root volatiles. <https://www.nature.com/articles/s41396-017-0035-3>
Estimating the economic costs of antimicrobial resistance. https://www.rand.org/pubs/research_reports/RR911.html
Oral application of inorganic phosphate protects mice from death. <https://www.sciencedirect.com/science/article/pii/S0039606008002869?via%3Dihub>
Application of a key signal molecule to the plant surface reduce bacterial entry and severity of disease. <https://mbio.asm.org/content/10/5/e01868-19.long>
Superbugs to kill 'more than cancer' by 2050. <https://www.bbc.com/news/health-30416844#:~:text=Drug%20resistant%20infections%20will%20kill,in%20700%2C000%20deaths%20each%20year.>

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By 2050, more people will die from antibiotic resistance than from cancer
<https://www.highnorthnews.com/en/2050-more-people-will-die-antibiotic-resistance-cancer-professor-refers-it-medical-climate-crisis>

Eleven industrial products that are derived from microbes.
<https://sciencesamhita.com/industrial-products-from-microbes/>

Global agriculture towards 2050.
http://www.fao.org/fileadmin/templates/wsfs/docs/Issues_papers/HLEF2050_Global_Agriculture.pdf

Status and trends in land and water resources. <http://www.fao.org/3/i1688e/i1688e03.pdf>

Glossary

Biofertilizer: Substance containing living microorganisms which, when applied to seeds or plants, promotes growth by increasing the nutrient supply to the host plant.

Biofilm: A consortium of one or more types of microorganisms that grow on surfaces.

Biopesticide: Type of pesticides derived from natural materials like animals, plants or bacteria. These can include live microorganisms that control pathogens and pests.

Biosensor: Analytical device for the detection of a chemical that typically combines a biological component with a physicochemical detector.

Biotransformation: Chemical modification made to a compound by an organism. In the context of this article, the production of added-value compounds by bacteria.

Microbiota: Ecological communities of commensal, symbiotic and pathogenic microorganisms associated with multicellular organisms like plants and animals. Microbiota include bacteria, archaea, protists, fungi and viruses.

Microorganism: Any microscopic organism which cannot be seen with a naked eye and that can live as single cell as well as in form of aggregates, colonies and biofilms.

Nitrogen fixation: Chemical process by which atmospheric nitrogen gas is transformed into ammonia or related nitrogenous compounds.

Nodule: Specialized organ developed on the roots of plants, mostly legumes, where symbiotic microorganisms fix nitrogen that can be subsequently used by the host plant to synthesize different compounds (e.g. amino acids, DNA, vitamins, etc.) required for growth.

***Pseudomonas aeruginosa*:** Pathogenic bacterium that causes diseases in plants, animals and humans. It can develop resistance to multiple antibiotics and the World Health Organization has included *P. aeruginosa* in a priority list of pathogens for which new antibiotics need to be developed.

Photoreceptor: Protein able to sense light and to trigger in response a signaling cascade.

Sensor protein: Protein that detects a physical or chemical stimulus to control different cellular functions.

Stomata: Pores in the plant leaf surface which allows movement of gases in and out of the plant. Stomata release compounds onto the leaf surface.

Symbiosis: Interaction between two different organisms living in close physical association, typically to the advantage of both.