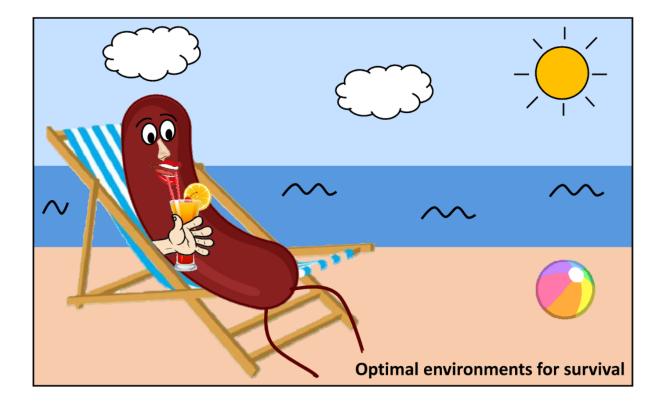
The key issue of gradients and bacterial taxis¹

Miss: How do microbes locate and select environments that are most favourable for their survival?



Tino Krell and Miguel A. Matilla

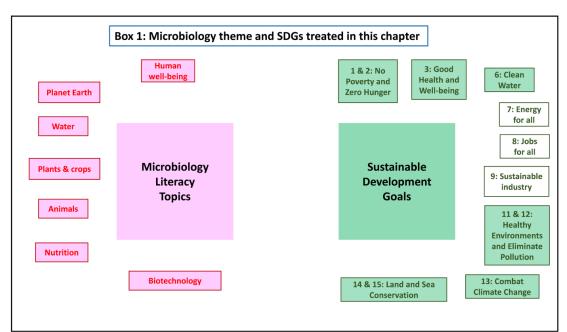
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¹ This has nothing to do with a taxi; microbial taxis means the directional movement of bacteria towards an attractant or away from a repellant

The key issue of gradients and bacterial taxis

Storyline

Let's place ourselves in a gastronomic fair. We are a large group of friends, each one of us with our own culinary habits and preferences. We go around the different food stands and, gradually, the group disperses. Based on our individual preferences for different smells, flavors, nutritional needs, or food intolerances, we each choose our favorite food stall. This is not unique to humans, as this reaction can also be observed in the microbial world. In humans, animals, plants, soils, rivers, seas or any other ecosystem we can find billions, even thousands of billions of microbes, per gram of soil or tissue, or per millilitre of water or biological liquids such as saliva. These microbes are highly adapted to live in specific ecological niches or hosts. They have selected and adapted to their favourite place. In many cases, they cannot survive outside these environments, which represents an extraordinary process of co-evolution between microbes and their environment or host. Likewise, the diversity of microbes in the different ecological niches as well as in their human, animal and plant hosts is extraordinary. There are very complex communities of microbes, and each member of the community may have its own preferences, intolerances and needs. In many cases, there are conflicts between different members of the community, leading them to compete for space and food. Many environments are not static and often undergo rapid changes. To efficiently survive in these highly competitive and dynamic environments, microbes sense different gradients of beneficial and harmful compounds, such as nutrients or oxygen for respiration, and move up or down the gradients to find optimal positions where they feel well: the environment which is best for them. This sensing occurs via hundreds of different sensor proteins (or receptors) that allow microbes to detect a high diversity of molecules, allowing them to efficiently select, colonize and adapt to their preferred niches and hosts. The knowledge of these microbial sensory capabilities can be exploited in strategies focused on the fight against pathogens or for the development of prebiotics to stimulate the growth and activity of specific bacteria with properties of interest (e.g. modulation of human, animal, plant microbiota, biodegradation of toxic compounds).



The Microbiology and Societal Context

The microbiology: microbial sensing of gradients of environmental compounds; chemoreceptors; microbial physiological and metabolic adaptations; chemotaxis; chemoattraction; chemorepellence; microbe-host symbiosis; microbial infection; prebiotics; plant, animal and human microbiota; anti-virulence therapy. *Sustainability issues*: No poverty and zero hunger; plant, animal and human health; clean water; healthy environments and eliminate pollution; climate change; land and sea conservation.

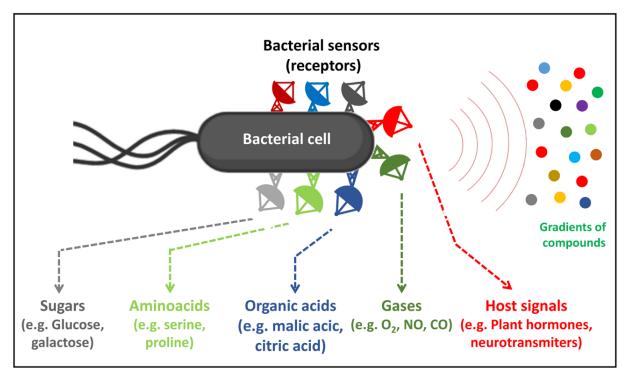
The key issue of gradients and bacterial taxis: the microbiology

1. *Life: a matter of gradients.* In life, we are constantly exposed to numerous physical and chemical gradients, often without even realizing it. For instance, we create a temperature gradient when we open the fridge, or we experience different levels of warmth depending on our distance from a fireplace. Light intensity changes throughout the day, influencing our circadian rhythm and sleep patterns. Atmospheric pressure decreases with altitude, and the experience of a concert varies greatly depending on whether we are in the front row or in the farthest stand. In the earlier example of a food fair, when you walk towards a food smell that you find particularly appealing, you are moving up an odor (olfactory) gradient: the closer you get to the source, the stronger the smell becomes. Similarly, as long as the stall is within your line of sight, the closer you approach, the clearer the stall and its delicious offerings become – you're also following a visual gradient.

The biosphere is full of such gradients, and many of them overlap. Microbes have evolved mechanisms to sense many of them. For example, pH levels can vary widely in different environments, such as the acidic conditions found in animal stomachs or certain soils. In salt ponds or hot springs, salt concentration and temperature, respectively, are also not uniform. Oxygen gradients are common in aquatic or soil environments, with oxygen being more abundant near the surface and decreasing with depth. Plant roots secrete compounds that serve as bacterial nutrients and the concentration of these compounds diminishes as we move away from the root. The ability to detect and respond to these gradients is crucial in microbial ecology and survival, influencing where microbes can thrive and how they interact with their surroundings and other (micro)organisms.

2. How do bacteria detect the presence of compounds from their environment? Sensors on the tongue, known as taste buds, allow different flavours to be detected and perceived. These taste buds are distributed on the surface of the tongue and contain sensory cells that are connected to nerve fibres that transmit the information to the brain. Similarly, bacteria have a high variety of receptors on their cell surface and in their cellular interior. Many bacteria possess hundreds of different receptors. Each of these can detect different types of compounds, for example, sugars, organic acids, amino acids, ions, aromatic compounds, among others.

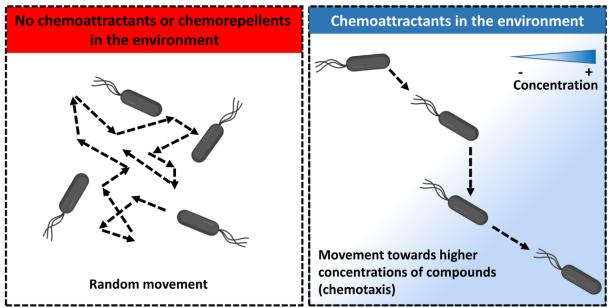
In many instances, these compounds can serve as a nutritional source for bacteria. In many other cases, these molecules can also provide bacteria crucial information about the surrounding environment. For example, through the detection of plant hormones and human neurotransmitters, bacteria can detect the presence of their hosts, permitting the activation of processes leading to their colonization or infection. Depending on the receptor type, compound binding to the receptor triggers a response that ultimately leads to various physiological and metabolic adaptations in the bacterium. These can include the activation of compound degradation, changes in motility, alterations in biofilm formation on both biological and nonbiological surfaces, or toxin production, among others.



The sensory capacity of bacteria. Bacteria have different sensory proteins (receptors) on their cell surface and inside the cell. These receptors act like radars, recognising specifically different types of environmental compounds, allowing bacterial responses to different concentrations of these molecules.

3. *Bacterial movement in compound gradients: chemotaxis.* Approximately half of all bacteria can swim. This activity is accomplished by the rotation of one or more long tail-like filaments called flagella (see image above), which are complex structures composed of at least 50 different proteins. Flagella are attached to the bacteria through what is known as the "flagellar motor", which can rotate at a speed of up to 100,000 revolutions per minute. This motor consists of a protein ring called the 'stator', which surrounds another protein ring that forms the 'rotor' to which the flagellum is attached, making the flagellar motor analogous to an electric motor. The movement of the flagellar motor is controlled by a sophisticated mechanism that does not respond to constant compound concentrations but rather to their gradients, allowing bacteria to recognise gradients and orient their swimming direction accordingly. Central to this mechanism are a particular type of receptors, termed chemoreceptors. Typically, chemoreceptors are located in the cell membrane and consist of two main components: an extracellular part that detects compounds and an intracellular region that triggers a bacterial response to the compound detected.

The detection of environmental chemical gradients by chemoreceptors, and the control of the activity of the flagellar motor and thus the direction in which the bacterium swims with respect to the gradient, constitutes the process called chemotaxis. Compounds detected by chemoreceptors can be either chemoattractants, which attract bacteria, or chemorepellents, which repel them. Frequently, chemoattraction occurs to nutrients, whereas chemorepellence permits in most of the cases to escape from toxic compounds. The ability of chemoreceptors to respond to gradients rather than constant compound concentrations is due to mechanisms that adjust their sensitivity to the ambient compound concentration through chemoreceptor methylation. This adaptation allows bacteria to efficiently respond to varying compound concentrations in three-dimensional space.



Chemotactic response of bacteria to gradients of compounds. In the absence of gradients of chemoattractants or chemorepellents, flagellated bacteria move randomly (left panel). As soon as they detect a chemoattractant, bacteria will direct their movement towards higher concentrations of this molecule (right panel). In the presence of chemorepellents, the process works the other way round, i.e. moving away from high concentrations of these compounds.

4. The relevance of chemotaxis in plant colonization and infection. Plants release different compounds – plant exudates – into the environment from their roots, leaves and other tissues. These exudates are complex mixtures that can contain thousands of different compounds, including sugars, amino acids, organic acids, ions, plant hormones, aromatic compounds, among others. Many exudate components can serve as nutrients and chemoattractants for microbes and influence the composition of plant-associated microorganisms (the plant microbiome). For example, roots release exudates that attract soil bacteria, triggering plant colonization and formation of the root microbiota. In fact, the density of bacteria in soil ranges from 10⁶ to 10⁹ bacteria per gram of soil, but in the vicinity of plant roots, these numbers can rise to 10¹² bacteria (1 trillion bacteria per gram).

Chemotaxis is often required for plant colonization by both beneficial and plant pathogenic bacteria. In fact, mutant bacteria that have been genetically modified so that they cannot perform chemotaxis, colonize plants at lower levels than the original (wild-type) bacteria. Employing similar genetic approaches, it has been shown that chemotaxis towards specific compounds such as amino acids, nitrate, organic acids and phytohormones is essential for colonization and infection of plants by bacteria. The relevance of chemotaxis to plant-associated bacteria is highlighted by the fact that, on average, such bacteria have more than twice the number of chemoreceptors of those that do not interact with plants.

5. Chemotaxis is important for the virulence of human and animal pathogens. Chemotaxis is a property exhibited by about half of human and animal pathogenic bacteria and, in many cases, has been shown to be crucial for their pathogenicity, which is indicative of the importance of this process for these pathogens. In fact, a high percentage of the pathogens highlighted by the World Health Organization as being most significant to public health perform chemotaxis. Chemotaxis appears to be particularly relevant for gastrointestinal pathogens. The gastrointestinal tract is a highly complex environment. To access it, microbes are typically taken in through the mouth and must transit the stomach, which is a very acidic and hostile

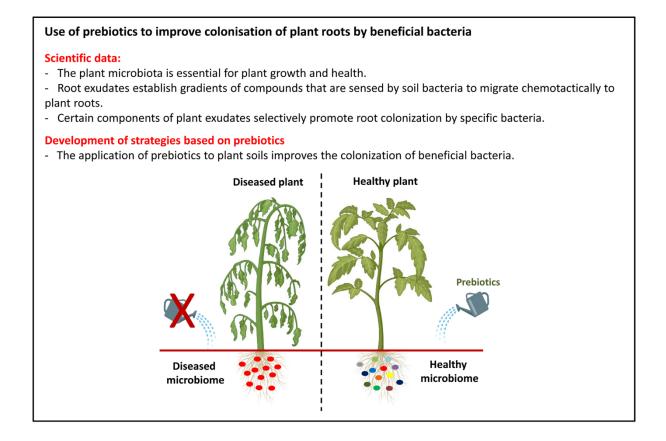
environment with a pH of 1.5 - 3.5. To facilitate survival in this harsh environment, many gastrointestinal pathogens employ chemotaxis to move to niches that are more favourable for their survival within the human/animal host. For example, the stomach pathogen *Helicobacter pylori* has several chemotaxis sensors that respond to pH changes. Elimination of these chemoreceptors in the bacterium prevents stomach colonization and disrupts the infective process. Chemotaxis also allows pathogens to migrate to wounds through recognition of compounds derived from damaged tissues, thereby promoting colonization and infection.

6. The ability of marine bacteria to sense gradients. The density of bacteria in marine waters varies depending on factors such as depth, turbidity and temperature. In general, bacterial density in surface marine waters is as high as 10⁶ bacteria per millilitre. However, bacterial density is strongly affected by nutrient availability. In fact, in marine waters, nutrients are not homogeneously distributed, and form gradients, permitting chemotaxis. Up to 20-fold variations in bacterial density have been measured over small distances (10 - 30 mm). Hence, the ability to perform chemotaxis in motile bacteria is a competitive advantage over non-motile bacteria, since it allows exploring greater distances and volumes of water 'space' in search of nutrients. The volume of water explored per day by a chemotactic bacterium is more than 10,000 times that of a non-motile bacterium, which moves passively or through Brownian movements.

Marine bacteria exhibit chemotaxis towards organic matter from phytoplankton and algae, as well as to exudates from photosynthetic bacteria. Fish feeding frenzies, such as those occurring on sardine runs (e.g. <u>https://www.bbc.com/future/article/20240520-how-south-africas-sardine-run-is-changing</u>), is an extreme example of the release of organic matter and creation of gradients. Chemotactic bacteria are frequently found associated with marine organism like corals, phytoplankton, protists and algae. This is indicative that exploring chemical gradients is an ecological advantage to establish interactions with other (micro)organisms, which are nutrient-rich hotspots in aquatic environments.

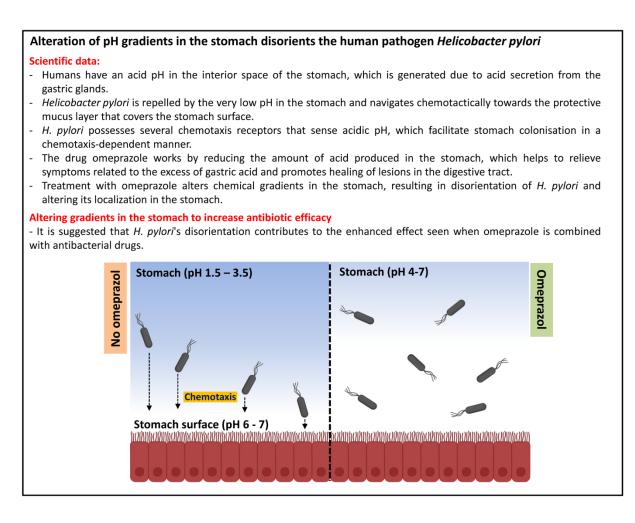
7. Prebiotics to select beneficial plant microbiota. Current predictions indicate that the world population will reach 10 billion by the year 2050, implying the need to increase agricultural production by more than 70% in the coming years to meet the increasing food needs of the world's population. The plant microbiota is essential for plant health and growth, providing protection against pathogens and assuring nutrients. In this context, the knowledge of the chemotactic capacities of beneficial (non-pathogenic) plant-associated bacteria will allow us to advance in the development of strategies to promote the development of plant microbiomes that promote plant health.

Plants recruit beneficial bacteria through a process in which chemotaxis towards root exudates plays a key role, particularly during the initial stages of plant colonization. Root colonization by beneficial bacteria was shown to promote plant growth and render the plant more resistant to pathogen attack. Metabolomics studies have identified compounds that are exuded by healthy plants at higher levels compared to those of diseased plants, which in turn is associated with changes in the root microbiome. This knowledge is of importance for the development and application of prebiotics – compounds with beneficial properties – to plant soils in order to favour the colonization by beneficial chemotactic bacteria. Alternatively, research has also allowed the development of genetically modified plants to alter their root exudation profiles and thus alter the composition of their root microbiota. These biotechnological strategies represent promising alternatives for increasing agricultural yields.



8. Blocking chemotaxis as a strategy to fight pathogens. Currently, our main weapons to combat human/animal and plant pathogenic bacteria is through the use of antibiotics and agricultural pesticides, respectively. Over the years, the application of antibiotics and pesticides has been crucial in saving millions of lives and sustaining high agricultural productivity by controlling plant diseases. However, their overuse and misuse are leading to the development of antibiotic- and pesticide-resistant pathogens, which have adapted to such treatments and no longer respond to them. This growing resistance poses a major challenge for both guaranteeing global health and sustaining agricultural production. In fact, current estimates, including those of the World Health Organisation, project that antibiotic-resistant bacteria will cause 10 million deaths annually by 2050.

An alternative to antibiotics is the use of anti-virulence therapies. Rather than killing pathogens, these therapies focus on inhibiting or neutralizing the virulence of microorganisms. Chemotaxis is among the bacterial processes that contribute to virulence, since it permits bacteria to move to sites or tissues that enable initiation of disease. Indeed, anti-virulence strategies that affect chemotaxis have been shown to disorient pathogenic bacteria, thereby leading to a reduction in the incidence of disease. To use a modern-day simile, this strategy can be compared to inhibiting bacterial GPS reception, which makes it more difficult to reach their destination. This inhibition of the bacterial GPS does not directly affect the viability of the bacteria, and so is less selective for acquisition of resistance than is the case with antibiotics that kill or inhibit the growth of pathogens, and may therefore prove to have longer utility. Future clinical strategies may be oriented towards the combined use of antibiotics and anti-virulence compounds.



Relevance for Sustainable Development Goals and Grand Challenges

- GOALs 1 and 2: No Poverty and Zero Hunger. Microbiota play a crucial role in plant health and growth. These microorganisms promote plant development through processes like nitrogen fixation, phosphate solubilisation, and plant hormone production, which enhance nutrient availability and stimulate root growth. Additionally, microbiota act as a protective barrier by outcompeting pathogens and producing antimicrobial compounds, thereby reducing disease incidence. An optimal microbiome enhances crop yield and resilience to adverse conditions. Many beneficial microbes colonize plants through chemotaxis, driven by plant-released compounds. Understanding this plant-microbe communication is key to modulating plant microbiota, ultimately increasing plant productivity an essential goal to support the growing global population.
- GOAL 3: Good Health and Well-being. The microbiota plays a vital role in human and animal health. This complex community of microorganisms is involved in numerous physiological processes that are crucial for maintaining health, including digestion and nutrient absorption (e.g. digestion of complex carbohydrates and fibers; synthesis and absorption of essential nutrients, such as vitamins B and K); immune system regulation and protection against pathogens; providing a protective barrier against pathogenic microorganisms. Indeed, an imbalance in the microbiota, known as dysbiosis, has been linked to metabolic disorders such as obesity, diabetes and cardiovascular diseases. A

recent analysis of over 200,000 genomes of human gut commensal bacteria has revealed a wide variety of chemoreceptors that are believed to play a key role in the survival and competition of these bacteria within the complex and competitive environment of the gastrointestinal tract. Additionally, chemotaxis is essential for a bacterium's ability to cause disease. Therefore, utilizing prebiotics to enhance the chemotaxis of beneficial microorganisms in human and animal microbiomes, while disrupting chemotaxis mechanisms in pathogenic bacteria, may promote the development of optimal microbiomes that reduce bacterial virulence.

- GOALs 6, 11 and 12: Clean Water, Healthy Environments and Eliminate Pollution. Industrialization, agricultural runoff, mining activities, and inadequate or outdated sewage systems are major contributors to water and soil pollution, posing significant threats to both environmental and human health. Bacteria play a vital role in combating this problem through biodegradation, permitting the breakdown of pollutants and contaminants. Certain bacteria exhibit chemotaxis towards toxic, biodegradable substances, increasing their accessibility and in turn degradation efficiency. This capability assists in the detoxification and cleanup of contaminated water and sites. Understanding how bacterial biodegraders detect and move chemotactically towards contaminants will provide a foundation for enhancing the effectiveness of future microbial-based technologies. Notably, the advancement of bacterial biosensors designed to detect specific pollutants holds promise for developing analytical tools that can rapidly assess water quality.
- GOALs 13, 14 and 15: Combat Climate Change, Land and Sea Conservation. Bacterial chemotaxis plays a critical role in environmental processes that are closely linked to climate change, such as regulating greenhouse gas emissions, nutrient cycling, ecosystem health and bioremediation. For instance, methanogenic bacteria, which produce methane as a metabolic byproduct, use chemotaxis to locate and metabolize organic matter. This chemotactic movement toward substrates directly influences methane production, a significant contributor to global warming. Similarly, denitrifying bacteria that produce nitrous oxide (N_2O) move toward nitrate or nitrite, precursors for N₂O, another potent greenhouse gas. The movement and activity of these bacteria in soil and aquatic environments can therefore impact nitrous oxide emissions. Moreover, both marine and terrestrial bacteria exhibit chemotaxis toward organic matter, which is essential for establishing and maintaining symbiotic and pathogenic relationships with other organisms. These interactions are vital for the carbon cycle, as bacteria break down organic matter from plants, animals, and other microbes, retaining some carbon in their biomass and releasing the rest as metabolites or CO_2 . Additionally, photosynthetic bacteria act as carbon sinks, capturing CO_2 from the atmosphere. These processes are crucial for ecosystem productivity and have significant implications for the global climate, as they influence both carbon sequestration and greenhouse gas emissions.

Potential Implications for Decisions

1. Individual

a. Have a balanced diet.

b. Avoid using antibiotics unless absolutely necessary.

c. Make appropriate use of soils, reducing their overexploitation and the use of chemicals (e.g. fertilisers, pesticides) in agriculture.

2. National Policies

a. Promote investment in basic research, which generates the knowledge base needed to solve important health, ecological and technological problems.

b. Reduce bureaucratic and legislative barriers to the use of microorganisms in sustainable agriculture, aiming to decrease reliance on chemical fertilizers and pesticides that threaten human, animal, and soil health.

c. Promote the use of genetically modified plants.

d. Progress in standards and regulatory frameworks to advance the use of microbiome-based therapies for human and animal health.

Pupil participation

1. Class Discussion

a. If you were a bacterium, to which parts of the human body or niches in the environment would you move by chemotaxis to gain access to many nutrients?

b. Can you think of scenarios where chemotaxis could give bacteria an evolutionary advantage over non-motile bacteria?

c. What might happen to bacteria if they couldn't move toward food sources?

2. Exercises

a. Search online and list various chemoattractants and chemorepellents detected by the human pathogen *Helicobacter pylori*.

b. List five species of human bacterial pathogens highlighted by the World Health Organization (WHO – see: https://www.who.int/publications/i/item/9789240093461). Then, visit https://mistdb.com/ to check if the selected bacterial species have chemoreceptors. Note that on the website "https://mistdb.com/" methyl-accepting chemotaxis proteins (or MCPs) are synonymous with chemoreceptors.

c. To visualize bacterial chemotactic speed, consider that some bacteria were found to move with a speed of 200 μ m/sec (or 0.2 mm/sec). First, determine the average length of a bacterium from online sources. Then, calculate the bacterial speed in terms of "times its length per second". Translate this speed into human terms by assuming an average human height of 1.8 meters (6 feet), and convert the speed to meters per second and kilometers per hour. Finally, compare this relative speed to Usain Bolt's record, who ran 100 meters in 9.58 seconds, to find how many times faster bacteria are compared to him relative to their size.

d. Game to help children understand how and why bacteria move towards certain stimuli (chemoattractants) or away from others (chemorepellents) by creating an imaginative scenario.

Materials:

- Paper and coloured pencils

- Tokens or small figures to represent bacteria
- Cardboard or large paper to draw a board.

Instructions:

Draw a board with a path for the bacteria to follow. Along the path, draw or place images representing chemoattractants (e.g., sugar, cereals) and chemorepellents (e.g., vinegar, alcohol). Have the children select tokens to represent bacteria and imagine a story where the bacteria are searching for "food" (chemoattractants) to grow and survive, while needing to avoid "dangers" (chemorepellents) along the way.

The Evidence Base, Further Reading and Teaching Aids

Marine microbes see a sea of gradients. <u>https://www.science.org/doi/10.1126/science.1208929</u>

WHO bacterial priority pathogens list, 2024: Bacterial pathogens of public health importance to guide research, development and strategies to prevent and control antimicrobial resistance. https://www.who.int/publications/i/item/9789240093461

The ecological roles of bacterial chemotaxis. <u>https://www.nature.com/articles/s41579-022-00709-w</u>

The effect of bacterial chemotaxis on host infection and pathogenicity. <u>https://academic.oup.com/femsre/article/42/1/fux052/4563582</u>

Accessing nutrients as the primary benefit arising from chemotaxis. <u>https://www.sciencedirect.com/science/article/pii/S1369527423000954</u>

Prevalence and Specificity of Chemoreceptor Profiles in Plant-Associated Bacteria. https://journals.asm.org/doi/10.1128/msystems.00951-21

Population - the United Nations. <u>https://www.un.org/en/desa/world-population-projected-reach-98-billion-2050-and-112-billion-2100</u>

International organizations unite on critical recommendations to combat drug-resistant infections and prevent staggering number of deaths each year. <u>https://www.who.int/news/item/29-04-2019-new-report-calls-for-urgent-action-to-avert-antimicrobial-resistance-crisis</u>

Bacterial chemotaxis in human diseases. https://www.cell.com/trends/microbiology/abstract/S0966-842X(22)00290-6

Framework for exploring the sensory repertoire of the human gut microbiota. <u>https://journals.asm.org/doi/10.1128/mbio.01039-24</u>

Chemotaxis to Hydrocarbons. <u>https://link.springer.com/referenceworkentry/10.1007/978-3-319-50542-8_43</u>

The Methanogenic Bacteria. <u>https://link.springer.com/referenceworkentry/10.1007/0-387-30743-5_9</u> The role of soil microbes in the global carbon cycle. <u>https://scijournals.onlinelibrary.wiley.com/doi/full/10.1002/jsfa.6577</u>

Ecology and Physics of Bacterial Chemotaxis in the Ocean. https://journals.asm.org/doi/full/10.1128/mmbr.00029-12

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

Glossary

Bacterial host: Refers to a living organism in which bacteria reside, thrive, and potentially establish a relationship.

Biodegradation: Process by which substances are broken down by the natural action of living organisms, typically used for toxic compounds.

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

Brownian movement: Refers to the random, erratic movement of microscopic particles suspended in a fluid resulting from their collision with fast-moving molecules in the fluid.

Gastrointestinal tract: Complex system of organs involved in ingesting, digesting, absorbing, and eliminating food and nutrients. It comprises the mouth, esophagus, stomach, small intestine, large intestine, rectum, and anus.

Helicobacter pylori: Pathogenic bacterium that primarily colonizes the stomach, responsible for gastrointestinal diseases, including ulcers and gastritis. It can develop resistance to multiple antibiotics and the World Health Organization has included *H. pylori* in a priority list of pathogens for which new antibiotics need to be developed.

Metabolomics: Large-scale study of small molecules, commonly known as metabolites, including their concentrations, interactions, and changes in response to various conditions.

Mutant bacteria: Bacteria that have undergone a change or alteration in their DNA, leading to a variation from the original genetic sequence.

pH: Scale that measures how acidic or basic a solution is. It ranges from 0 to 14, with values below 7 indicating acidity and values above 7 indicating basicity.

Photosynthesis: Process by which green plants, algae, and some bacteria convert light energy, water and carbon dioxide into chemical energy, specifically glucose, which they use for growth and metabolism.

Phytoplankton: Free-floating photosynthetic microorganisms in aquatic environments that are a crucial component of the aquatic food web.

Prebiotic: Compounds that selectively stimulate the growth and activity of beneficial microorganisms.

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

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.

Niche: Combination of environmental conditions, resources, and interactions with other species that enable a species or population to survive, grow, and reproduce within a particular ecosystem.

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