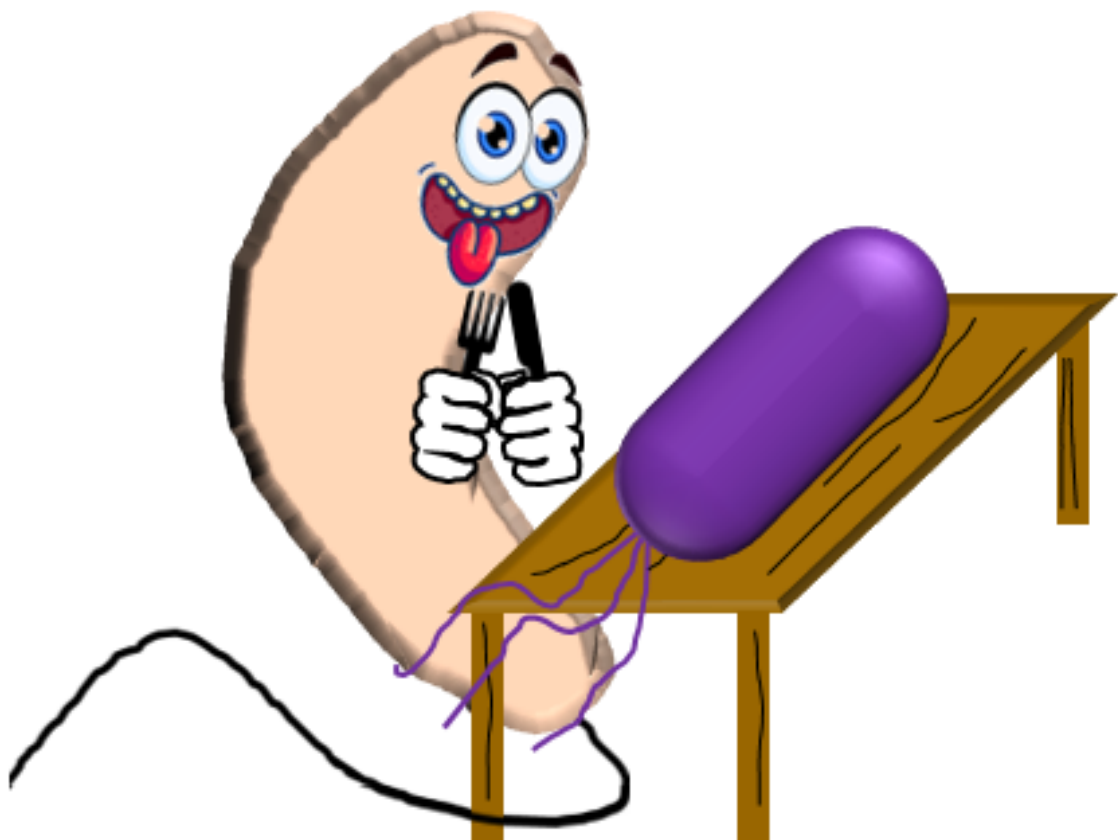


**Predatory bacteria
Carnivorous? Herbivorous? Bacteriovorus!**

What Do Predatory Bacteria Find Delicious?



Wonsik Mun and Robert J. Mitchell

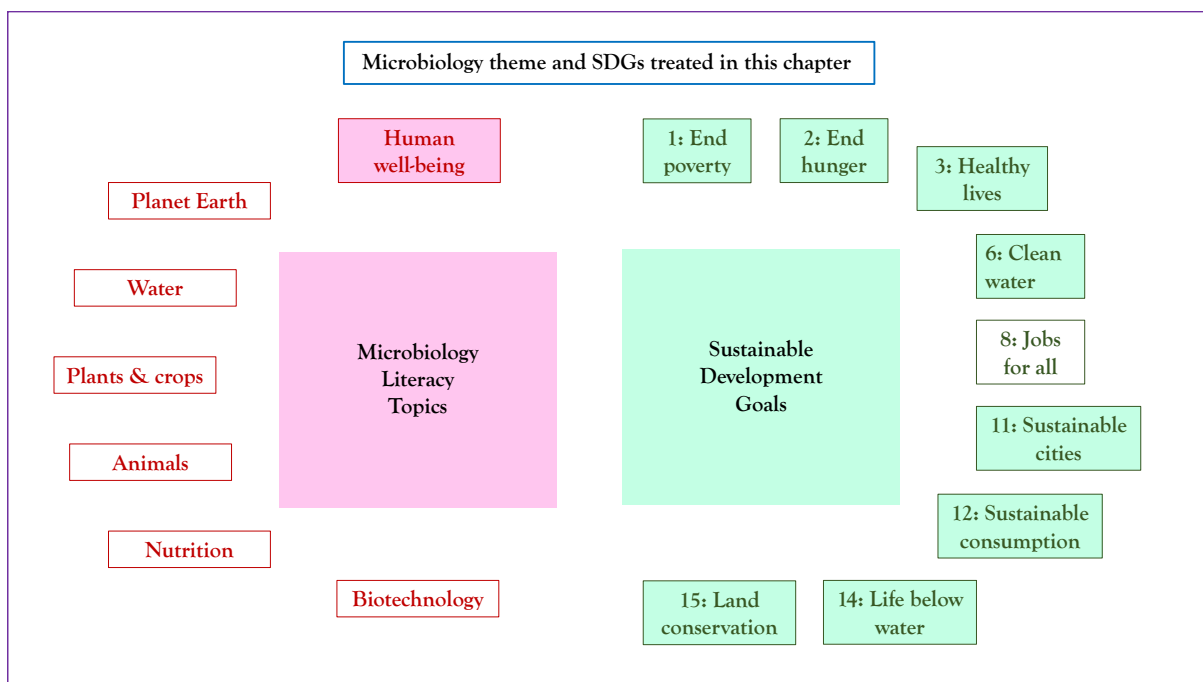
Predatory bacteria

Storyline

For the public, the general perception of bacteria is typically a negative one, and one that is reinforced by advertisements offering products that kill 99.9% of germs. While bacterial pathogens do exist and are a problem, they often represent only a very small percentage of all bacterial species within a given environment. One thing many people do not realize, even many microbiologists, is that some bacterial species act like pathogens towards pathogenic bacteria but do not harm us, plants or animals. These bacteria are commonly referred to as “predatory bacteria” and can be viewed as a classic case of “the enemy of my enemy is my friend”. Along these lines, scientists around the globe are considering different ways that predatory bacteria and their enzymes can be used in a beneficial manner. Predatory bacteria and their activities, thus, have multiple consequences for Sustainable Development Goals.

The Microbiology and Societal Context

The microbiology: microbial predation; microbial diversity and location; pathogens; enzymes. And, *peripherally for completeness of the storyline:* antibiotic resistance. *Sustainability issues:* poverty; health; food and energy; environmental pollution; sustainable cities, marine and terrestrial environments.



Predatory Bacteria: the Microbiology

1. ***Bacteriovorus? Predatory bacteria!*** Microbes have various strategies to eat and grow. Some bacteria have developed a very special method, which is to eat other bacteria. We call this 'bacteriovorus', and such bacteria are called predatory bacteria. Predation is usually thought of as big eating small but, in this case, it is actually small eating big. A well-known example of predatory bacteria is BALOs (Bdellovibrio-and-Like Organisms). BALOs are Gram-negative bacteria (meaning they have two membranes on the outside of the cell), that eat other bacteria, and they do this by penetrating into space between the two membranes (the periplasm) of other Gram-negative bacteria and releasing various hydrolytic proteins therein. These proteins, *i.e.*, enzymes, break down the proteins and nucleic acids (DNA and RNA) of the prey into their component monomers, *i.e.*, individual subunits of amino acids and nucleotides, which are then used for the growth and replication of the BALO bacterium.



Bdellovibrio on the attack. Left (rod shape with a long flagellum) - *Bdellovibrio*, Right (dented shape) - *Acinetobacter baumannii*. Image generated by AEMLab, UNIST

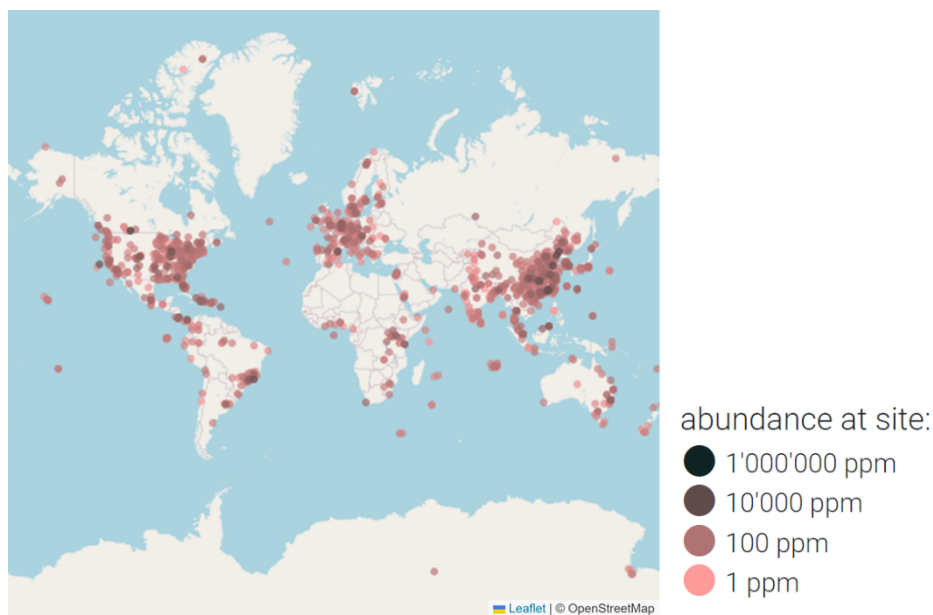
2. ***A variety of predatory bacteria exist in nature.*** How diverse are the predatory bacteria? We may think that predatory bacteria are unfamiliar and rare but, in fact, they are very common. And their diversity also extends to their predation mechanisms. As mentioned above, BALOs generally use an intraperiplasmic (between the two membranes) mode of predation, but others use different predation schemes. For instance, *Vampirococcus*, which, as its name suggests, attaches to the outside of the prey like a vampire and “sucks” the nutrient out of its prey cell. Another common group of predators is represented by *Myxococcus*, which uses a wolf-pack attack where several predators surround a prey and secrete hydrolytic proteins, causing the prey to burst open. Finally, there is *Daptobacter*, a predator that penetrates all the way into the cytoplasm of its prey.

In some cases, predatory bacteria are known to “sniff out” their prey and move with terrifyingly high speeds (like a cheetah) to chase after their prey.

Despite their diversity, predatory bacteria have one key trait in common: they all break down prey macromolecules (DNA, RNA, proteins, etc.) and transform them into a form that can be “eaten” by the predator.

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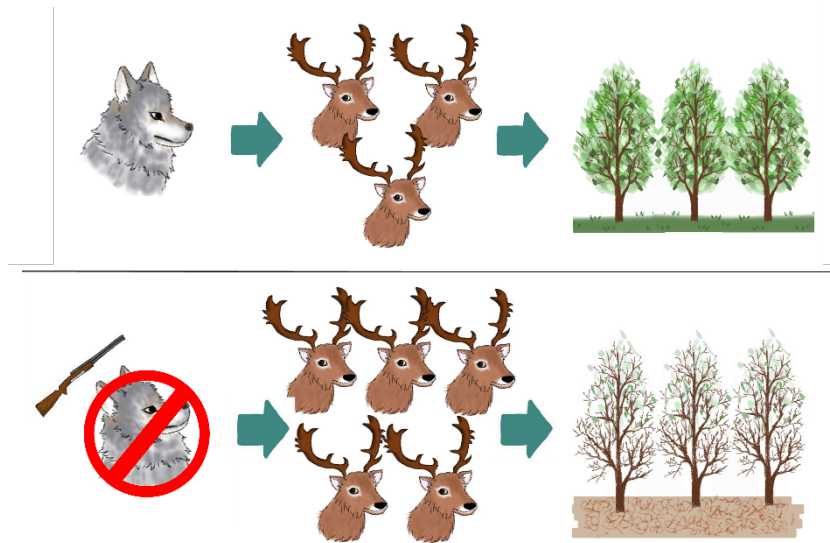
3. ***Predatory bacteria have been found almost everywhere on Earth.*** Where do predatory bacteria live? Just as they are diverse, their habitats are also very diverse. Surprisingly, you likely encounter predatory bacteria every day. Predatory bacteria exist pretty much everywhere that bacteria can be. For instance, not only were predatory bacteria isolated from soil, rivers, water reservoirs, the ocean, seas and even wastewater treatment plants in temperate (warm) climates, but also were found at low temperatures, such as in the Arctic and Antarctic, and high temperatures, such as in Himalayan hot springs. Moreover, do you know that many bacteria live in your gut? Some of these are “eaten” by predatory bacteria. So it should come as no surprise that predatory bacteria have also been found in the guts of animals and humans, as well as in a number of other locales where bacteria are present, such as the gills of crabs, sponges, plant rhizosphere (roots) and so on. Predatory bacteria are clearly an important component in the world's microbial consortia!



Microbe Atlas, Abundance of *Bdellovibrio bacteriovorus* in the world (Accessed Nov. 14th. 2022).
https://microbeatlas.org/index.html?action=taxon&taxon_id=90_271;96_2688;97_3301;98_4155

4. ***Predatory bacteria influence the diversity of microbial communities.*** So predatory bacteria are everywhere, but what role do they play in nature? The role of predators in nature has not yet been clearly elucidated. However, the role of animal predators is pretty well understood. Have you ever visited Yellowstone National Park in the United States? It is famous for elk. However, wolves also live there and they eat the elk. They were also threatening the tourists who came to the park, so the park ranger cleared the wolves. Everyone expected a peaceful and prosperous park environment, but in reality, devastation began after the wolves disappeared. It was temporarily an elk's paradise but, after a while, the growing elk population ate much of the plants and, as a result, other herbivores started dying out and the ecosystem diversity began deteriorating. Only after the wolves were reintroduced to the park did they see ecosystem diversity restored.

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(Drawing by ©Yerin Hong)

While you may think a predator will decrease diversity because it eats members of that ecosystem, the example above with the wolves and elk, shows that this is not necessarily the case because the elk became a greater problem that was not controlled without the wolves. Similarly, studies have shown that predatory bacteria are also related to ecosystem diversity, but on the microbial level. Researchers have found the microbiomes of various hosts (the bacteria within the insect or animal, including nematodes, fruit flies, hydras, sea anemones, sponges, dogs and humans) were more diverse (so, healthier!) when they had greater numbers or different types of predatory bacteria within them. Further, other researchers introduced predatory bacteria into the gut of rats and several beneficial bacterial species increased in number. While more studies need to be done, both of these findings strongly suggest predatory bacteria provide a beneficial influence on the diversity of bacterial community composition. As biodiversity is a key attribute of stable ecosystems everywhere, not just in our gut, it is critical that we discover and understand the roles predatory bacteria play in nature.

5. ***The various enzymes produced by predatory bacteria are excellent research resources.*** The enzyme industry is a huge entity that is constantly expanding. Enzymes are used in a variety of applications, such as in laundry detergents, medical treatments, feed additives, digestive agents, chemical and food processing, leather processing, pharmaceuticals, biotechnology and so on. As mentioned above, predatory bacteria produce and utilize numerous hydrolytic enzymes to break down the macromolecules within prey bacteria into edible monomers. As an example of this, the predatory bacterium *B. bacteriovorus* HD100 encodes for 15 lipases (enzymes which digest fats), 10 glycanases (which break down complex sugars), 150 proteases/peptidases (to hydrolyze proteins), 20 DNases and 9 RNases (digests the prey DNA and RNA, respectively) in its genome. This variety of enzymes is produced by a single predatory bacterium, meaning with all the diverse predatory bacteria already found, many more are also available. For enzyme research, it is important to collect various candidate proteins, and predatory bacteria are clearly a very good research subject for enzyme discovery.

This is all the more true as we turn our attention to predatory bacteria from diverse environments, expanding their potential research resources even further. All bacteria are adapted to their environment and produce special enzymes that help them survive. Using this fact, we

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can obtain and utilize special enzymes that work under harsh conditions. One example of this that has been tremendously helpful for scientists and companies alike is DNA polymerase, an enzyme that synthesizes DNA. While this enzyme is found in all bacteria, for use in the polymerase chain reaction (PCR), where it amplifies small pieces of DNA, it must remain active at high temperatures. To address this, researchers use Taq DNA polymerase, an enzyme that was isolated from *Thermus aquaticus*, a microorganism that lives in hot springs and grows best when the temperature is 65–70 °C (149–158 °F). As noted above, predatory bacteria were also found in extreme environments, such as hot springs (>50 °C) and in cold regions, like the Arctic and Antarctic. Much like *T. aquaticus*, it is likely that we can discover excellent enzymes by isolating and studying predatory bacteria that are adapted to these environments.

6. ***Predatory bacteria are promising alternative antibiotics.*** Antibiotics are a savior for mankind to treat bacterial infections. However, antibiotic-resistant bacteria are rapidly spreading due to many different contributing factors. These are called superbugs, particularly when the pathogen is resistant to multiple antibiotics at the same time. Therefore, new or substitutes for antibiotics are essential.

Predatory bacteria have the potential to be used as alternatives to antibiotics. One reason for this is predatory bacteria can prey on and eliminate various pathogens (including multidrug-resistant pathogens). For example, one predatory bacterium, *B. bacteriovorus* HD100, can “eat” over 100 different human pathogens, including *Salmonella* and *E. coli*. Furthermore, they are excellent biocontrol agents in that they not only kill pathogens but also destroy the pathogen’s DNA and toxic proteins. Furthermore, only a small number of studies have reported resistance to predatory bacteria, meaning they may remain active for years to come, especially since there are so many varieties of predatory bacteria.

By the way, wouldn't BALOs be useless if they were harmful to the human body? We do not have to worry about this! Many groups around the globe have investigated potential effects of predatory bacteria on human cells and various animals and they all found predatory bacteria to be non-toxic. In contrast, administration of predatory bacteria to pathogen-infected animals (mice, rats, chicks, zebrafish, etc.) reduced the pathogens and alleviated disease symptoms, meaning we may be able to use BALOs to treat bacterial infections in humans. Research is actively being conducted to develop predatory bacteria as substitutes for antibiotics so, hopefully in the near future, a doctor may prescribe predatory bacteria at the hospital, maybe even in a form like yogurt. Wouldn't that be cool?

Relevance for Sustainable Development Goals and Grand Challenges

- **Goal 1. End poverty in all its forms everywhere.** Bacterial infections and deaths are highest in poverty-stricken countries and regions. Premature deaths from infections also make it difficult for people in these areas to overcome poverty. Medicines are generally not cheap, limiting the number of people who have access. Moreover, as antibiotics become less effective due to the spread of resistance, impoverished nations and communities are likely to be the hardest hit. Predatory bacteria and their activities can mitigate many of these problems as they are cheap to produce, making them more readily available than chemical drugs, and they are active against pathogens that are resistant to antibiotics.
- **Goal 2. End hunger, achieve food security and improved nutrition and promote sustainable agriculture.** Eco-friendly methods for sustainable agriculture/aquaculture

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are currently in the limelight and represent methods to reduce environmental pollutants (such as antibiotics and herbicides) and replace them using animals or bacteria. Similar studies have shown the benefits of using predatory bacteria as biocontrol agents in agriculture or aquaculture. For example, *Myxobacteria* was used to prevent cucumber wilt while *Bdellovibrio* was used to prevent white feces syndrome, a fatal disease in shrimp farming. As such, predatory bacteria represent a new eco-friendly method for effective and sustainable food production.

- **Goal 3. Ensure healthy lives and promote well-being for all at all ages.** COVID-19 infected more than 600 million people worldwide and killed more than 6.6 million by the end of 2022. This pandemic illustrated how diseases without cures can greatly affect our lives on a global scale. For nearly a century, bacterial infections have been treated with antibiotics but resistance is spreading and approaching a serious level, one where antibiotics may not be available in the near future. As a countermeasure to this, alternatives to antibiotics have garnered attention, with predatory bacteria one such promising alternative. Worldwide, studies are constantly underway to evaluate how best to utilize predatory bacteria as antibiotic alternatives and, if successful, they will help protect our future.
- **Goal 6. Ensure availability and sustainable management of water and sanitation for all.** The availability of water resources is a major issue world-wide, with many people living with a limited quantity of water and many more lacking access to clean water. While predatory bacteria may not be able to reduce all microbes in groundwater, they are active against many gut bacteria and, in particular, intestinal pathogens. Consequently, the addition of predatory bacteria to unpurified water has the potential to reduce the spread of water-borne bacterial diseases and prevent downstream outbreaks.
- **Goal 11. Make cities and human settlements inclusive, safe, resilient and sustainable.** Amongst the municipal solid wastes produced by cities, microplastics are a growing problem with serious far-fetching impacts on the environment. Since plastics do not decompose rapidly, appropriate treatments, such as recycling or biodegradation, are essential. Two methods to achieve this are the use of plastic-degrading bacterial strains or enzymes engineered for this purpose. Likewise, predatory bacteria also possess enzymes to degrade a biodegradable plastic (polyhydroxyalkanoate) but, as many of their enzymes have not yet been studied, it may be possible to find something else that is also useful.
- **Goal 12. Ensure sustainable consumption and production patterns.** Pharmaceutical companies use microbes to produce and synthesize many of the drugs that they sell. As predatory bacteria require bacterial hosts for their growth, the resources needed for their production (non-pathogenic bacteria) are already available as wastes from these other processes.
- **Goal 14. Conserve and sustainably use the oceans, seas and marine resources for sustainable development.** Using predatory bacteria as an antibiotic or agricultural substitute also protects aquatic ecosystems and organisms as less antibiotics and herbicides (or other harmful substances) are used. This would reduce their runoff and entry into water system. While more predatory bacteria will enter these waterways, they are already members of the natural environment, meaning there is minimal risk for harm.

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- **Goal 15. Protect, restore and promote sustainable use of terrestrial ecosystems, sustainably manage forests, combat desertification, and halt and reverse land degradation and halt biodiversity loss.** The stability of the environment is integrally related with its biodiversity. While reckless development or environmental pollution can wreck lands, making them uninhabitable, examples of bioremediation using microorganisms (fungi, yeast, and bacteria) to restore ruined environments have also been demonstrated. Within soils, the majority of organisms are bacteria and their presence and diversity within this environment affect the health of other organisms, including plants and animals. As a member of a useful microbial community, predatory bacteria may be utilized to help regenerate and maintain soil biodiversity and vitality.

Potential Implications for Decisions

1. Individual
 - a. Being exposed to bacteria - is it always harmful? Are all bacteria bad?
 - b. Impact of antibiotic usage (on your gut, on your garden, etc). What is/are the best properties of antibiotics in terms of diversity or antibiotic resistance?
2. Community policies
 - a. Local environmental consequences (pathogens and antibiotic resistance), provision of clean drinking water
 - b. Health costs associated with multidrug-resistant pathogens
 - c. Non-microbial parameters: support of various industries (detergent, food preparation, etc) and third-world communities (cheap production)
3. National policies relating to predatory bacteria
 - a. Healthcare economics of bacterial diseases
 - b. Environmental pollution
 - c. Ensuring safe drinking water supplies

Pupil Participation

1. *Class discussion of the issues associated with predatory bacteria*
 - a. Being exposed to bacteria - is it always harmful? Are all bacteria bad for us?
 - b. Impact of antibiotic usage (on your gut, on your garden, etc). What is/are the best properties of antibiotics in terms of diversity or antibiotics resistance?

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2. Pupil stakeholder awareness

- a. Predatory bacteria have several positive consequences for the SDGs. Which of these are most important to you personally/as a class?
- b. Bacteria are often viewed as being icky or undesirable. What ways are bacteria beneficial to you and your health? (Hint: gut health or yogurt)
- c. Can you think of other places that you can use predatory bacteria to remove pathogens?

3. Exercises

- a. Several possible applications of predatory bacteria were presented in this chapter. Can you think of any other place where you think they would be helpful?
- b. One highlighted area discussed in this chapter was the use of specialized proteins in your daily life. Can you think of other areas where bacterial proteins have benefited you and your families?

The Evidence Base, Further Reading and Teaching Aids

Predatory bacteria

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Glossary

amino acids: subunit (monomer) components used by cells to synthesize their proteins.

antibiotic-resistant: Term given to bacteria that display resistance to antibiotics. This resistance decreases the efficacy of the antibiotic drug and either requires a higher dose or an alternative antibacterial/antibiotic to treat the pathogen.

bacteriovorus: term meaning “bacteria eater”. This spelling is used primarily as the species name for strains of *Bdellovibrio bacteriovorus*, a predatory bacterium.

BALOs: *Bdellovibrio*-and-like organisms. This acronym is generally used to refer to predatory bacteria that possess the obligate, intraperiplasmic lifestyle seen in *B. bacteriovorus*.

biocontrol agents: Living organisms (or their derivatives) that can be used to control or remove other unwanted organisms from a given ecosystem. They, in effect, help control the presence of harmful organisms and/or their negative impacts.

biodiversity: This term refers to how much diversity exists within a given ecosystem. When biodiversity is high, it means the number of different organisms (microbes, plants, insects and animals) that live and interact in that ecosystem is high, while low biodiversity refers to the opposite case. Ecosystems can be anything from ponds and forests to the human gut. A sustained, long-term high biodiversity in a given ecosystem is generally viewed as being environmentally beneficial.

cytoplasm: The inside of a cell containing DNA and RNA, as well as many proteins. It is located within the cytoplasmic membrane.

DNA polymerase (Taq): Enzyme that replicates the DNA molecule within cells. This protein is critical in replicating the genome of bacteria when they are dividing. The *Taq* DNA polymerase is from a heat-resistant microbe, *Thermus aquaticus*, making it a very useful component in PCR reactions to amplify small DNA segments.

DNase: Enzymes that hydrolyze DNA molecules into their component nucleotide monomers for the bacterium to use as an energy source.

ecosystem: The collective grouping of all living and non-living components within a given environmental setting. All of these components interact with one another and determine the stability and health of their environment. Ecosystems can be anything from ponds and forests to the human gut.

enzymes: These are proteins with catalytic functions, meaning they act on and change a substrate to a product.

genome: The complete set of DNA within an organism. It contains all of the genes present in

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and encodes for all of the proteins and enzymes produced by that organism.

glycanases: Hydrolytic enzymes that help degrade complex sugars, reducing them to their cognate sugar monomers for the bacterium to use as an energy source.

Gram-negative: A major group of bacteria whose members all have two cellular membranes. The first is the cytoplasmic membrane, which separates the bacterial proteins from the environment. The second surrounds the first and is called the outer membrane. Between these two membranes is the periplasm.

herbivores: Animals that consume only vegetation, *i.e.*, plants, for their survival. Examples of herbivores are rabbits, deer and elk.

hydrolytic: Possessing the ability to cleave other molecules. Hydrolytic enzymes are proteins that can insert a water molecule into a chemical bond, splitting the molecule in the process. This term is used when describing the breaking down of polymers into their cognate monomers, such as proteins (polymer) into amino acids (monomer) or DNA/RNA (polymer) into nucleotides (monomer).

intraperiplasmic: Within the periplasm. This term is used to describe predatory bacteria during the predation cycle after they entered the periplasm of their prey.

lipases: Hydrolytic enzymes that help degrade lipids and fats.

macromolecules: Also referred to as polymers, macromolecules are large molecules formed by connecting monomers together like beads on a chain. Examples of macromolecules including proteins, DNA and RNA molecules.

multidrug-resistant pathogens: Pathogenic bacterial strains that are resistant to many different antibiotics, reducing the possibility of treating them using conventional antibiotic therapies. Also referred to as “Superbugs”

monomer: Individual subunit that, when combined together, form large compounds called polymers. Examples of monomers include nucleotides and amino acids.

nucleic acids: DNA and RNA. Polymers of nucleotides within a cell which encode the information for all of the proteins produced by that organism.

nucleotides: subunit (monomer) components used by cells to synthesize their nucleic acids.

pathogen: a microbe able to cause an infectious disease

periplasm: This is the space between the two membranes in Gram-negative bacteria. Within the context of BALOs

polymer: A large molecule formed by connecting monomers together. Examples of polymers include the macromolecules in all cells, including proteins, DNA and RNA molecules.

predatory bacteria: These bacteria attack and consume other bacterial cells. While some are opportunistic predators, meaning they can attack other bacteria under certain conditions (like starvation), others are obligate predators, meaning they can only grow and reproduce by preying on another bacterial cell.

proteases: Hydrolytic enzymes that digest proteins, reducing them to their cognate amino acid monomers for the bacterium to use as an energy source.

rhizosphere: Region located around the plant roots. The rhizosphere tends to have many plant-associated bacterial species present within it that live in close relation with the plant.

RNase: Enzymes that hydrolyze RNA molecules into their cognate nucleotide monomers for the bacterium to use as an energy source.

superbugs: General term used to define pathogens that are resistant to many different antibiotics, reducing the possibility of treating them using conventional antibiotic therapies.

toxic: Possessing any damaging property (chemical or biological) that causes harm to other organisms. Generally used when referring to a detrimental organism or compound.

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wolf-pack: Predation mechanism used by some predatory bacterial species, especially *Myxobacteria*. These predatory bacteria surround their prey, much like a pack of wolves, thus their descriptive, and secrete hydrolytic enzymes that degrade the prey. The nutrients released from the “digested” prey are then consumed by the wolf-pack members for their growth and development.

wastewater treatment plant: A facility in which a combination of various processes (e.g., physical, chemical and biological) are used to treat municipal and industrial wastewaters and remove pollutants, including pathogens and antibiotics.