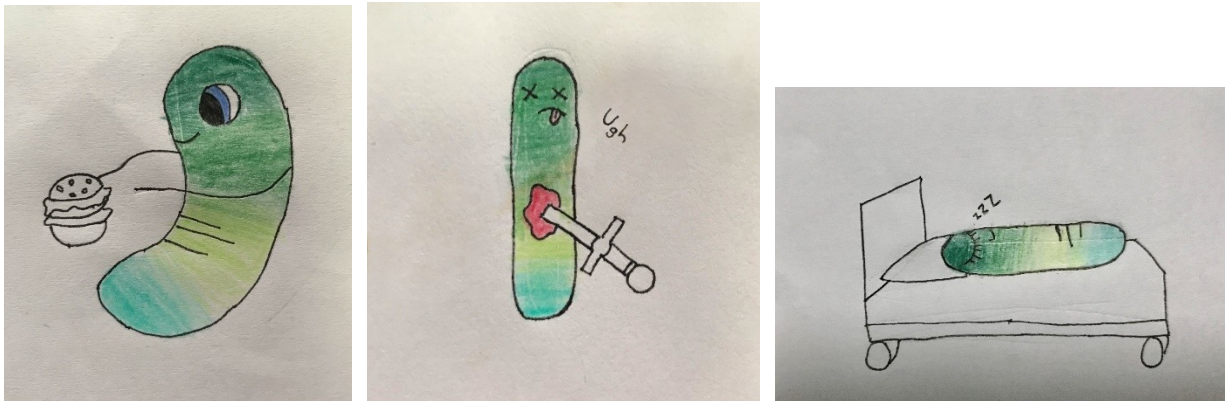


Life, Death, and Suspended Animation

*Mum: teacher told us today that we are living longer all the time:
how long do microbes live?*



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Storyline

Theoretically, microbes can be immortal. However, to borrow from Thoreau, most microbes lead lives of quiet desperation, so they sleep. Thus, in contrast to what is widely held, rather than growing rapidly, most bacteria spend the bulk of their existence, growing very slowly, if at all. Surprisingly, the vast majority of bacteria are found in ocean sediments and in the continental subsurface (not in lakes and animals) where they hardly grow at all: these cells may double every 10 to 100 years and are essentially in a state of suspended animation; i.e., they are dormant. Of course, if given nice food like sugar water, many bacteria like *E. coli* from our gut, can double in 20 minutes, which means they can increase their numbers rapidly (i.e., exponentially). Given limitless food, a single *E. coli* cell can grow exponentially into a mass of cells that will reach the weight of the Earth in a little less than two days (44 hr) and reach the mass of Jupiter in three hours' more time. But of course, bacteria do not reach these levels since they run out of food. This is true for microbes in the environment and in us. Therefore, for food safety and to curtail infections, it is important to be able to differentiate microbes merely in suspended animation from dead ones, and to recognize how bacteria wake. This requires that we describe traits for cells undergoing growth, for those in suspended animation, and for those that are dead, as well as indicate what resuscitates drowsy microbes.

Life, Death, and Suspended Animation: the Microbiology

Live vs. Dead vs. Sleeping Cells

1. ***Microbes can grow rapidly or be in suspended animation.*** Microbes propagate by binary fission – a cell grows in size and then, once a critical size is reached, it divides into two daughter cells – and can live forever by continually dividing. When presented with nutrients, bacteria can grow rapidly, i.e., exponentially (2 cells become 4 which become 8, and so on), until they run out of food or until toxic wastes accumulate and inhibit their growth. Different bacteria grow at different rates, depending on their food source and the compounds they use to get energy. For example, the best-characterized bacterium, *Escherichia coli*, grows by doubling every 20 minutes when given its favorite foods, like sugars and amino acids and when given oxygen.

In contrast, some microbes known as the archaea grow very slowly, even under their ideal conditions; these microbes live in communities in ocean sediments, where there is no oxygen present that is needed for fast growth. Unlike fast-growing *E. coli*, these ocean soil dwellers double every nine months, since they grow on methane gas, which is hard to capture compared to sugars dissolved in water.

One fascinating feature of microbes is that they can be put in **suspended animation**: scientists frequently store bacteria in very cold freezers (-80°C) where the bacteria will last forever, unchanged.

2. ***Microbes have three flavors: live, dead, or dormant.*** One key feature of living cells is that they are organized and are able to create energy, since making the building blocks of life, like DNA and protein, requires energy. Therefore, **live cells** are characterized by having intact membranes that protect their cellular contents – the cytosol – from the outside world and that are used to make energy. Microbes (and our cells) are very clever and embed in these

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membranes enzymes that make energetic compounds like ATP. Inside these membranes, the cytosol contains the ribosome machinery to convert the information encoded in DNA into protein and the few thousand other biochemical compounds of living microbes. Moreover, the cytosol of the healthy cell maintains proteins in a correctly-folded state and keeps DNA undamaged. In contrast, the hallmarks of dead cells include compromised membranes, damaged DNA, and denatured proteins that form aggregates. Also, some dead cells are empty membrane sacks whose cytosol containing the ribosome machinery and genetic material has leaked away. Therefore, a working definition for cell death is the inability to grow in the presence of nutrients. Since most microbial cells are starving or undergoing stress, they may enter a sort of suspended animation; i.e., a dormant state, known as spores or persistence, until food arrives and the stress ends. This dormant state protects the cells from myriad insults. Spores are formed by Gram positive bacteria (those with a single membrane surrounding the cytosol) and consists of durable armor that protects cells from myriad environmental insults (e.g., heat, acid, drought, radiation). The resting state of Gram negative cells (those with two membranes surrounding the cytosol) lacks the armor of a spore and is known as persistence. Gram positive bacteria can also become persistent. Persister cells have low energy (ATP) levels and have mothballed their ribosome machinery so they cease making protein. These dormant states may protect bacteria for years.

3. ***“No microbe is an island” and their homes are slime.*** In us and in Nature, anywhere there is liquid water, microbes make their homes. Most surprising, bacteria do not prefer to live alone, but instead, like us, they thrive in communities where they communicate to one another. The homes of microbes are called biofilms, due to their film-like nature and their common characteristic of sticking to solids, such as to our teeth in our mouths and to rocks in rivers and shells in oceans. The building blocks of microbial homes are primarily sugars, proteins, and oddest of all, DNA. Now you may be familiar with DNA as being a string genes that code for proteins, but in microbial homes, DNA is used as a glue that helps hold the bacteria in place. In biofilm homes, the communication devices are not ‘cell phones’ (beware of puns!) but instead are chemical signals known as quorum sensing signals. This communication method allows microbes to sense how many other cells are in their neighborhood and decide whether or not to build the biofilm. Just like our homes, biofilms protect microbes from harsh conditions, such as temperature fluctuations and from chemical threats, like antibiotics. Also, biofilms protect microbes from predators, such as protozoa, which eat bacteria, as well as protect the cells from our body’s anti-infection systems, white blood cells, which seek and eat microbes.

In good times, like any inspired builder/developer, some microbes of the community leave the biofilm in search of more territory to conquer and cover with slime. This requires the building blocks of the biofilm to be disassembled, by enzymes, which eat the DNA and sugar building blocks, thereby ungluing some of the microbes in the biofilm so that they can leave. Also, when times are hard (i.e., the microbes in the biofilm run out of food), some members of the community will decide it is ‘better to travel’ and leave the community in search of greener pastures.

Because biofilms are a film of about 100 cells thick, microbes in different positions experience different conditions. Therefore, the cells closest to nutrients can grow rapidly whereas the cells at the bottom of the heap may not grow at all and are dormant. So, like any realtor knows, it is location, location, location that matters in a biofilm.

4. ***Where microbes live.*** Microbes are abundant on Earth and live primarily in three main habitats: in ocean subsurfaces (35%), in soil and its subsurfaces (55%), and in ocean water (10%). Less than 1% of all microbes on Earth live in fresh waters, and you may be surprised

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that microbes may be found in the atmosphere. In fact, rainwater contains microbes, and microbes may provide nuclei for cloud and ice/snow formation. The way microbes live in these habitats is primarily in biofilms.

Microbes also live on and in us as each of us carries about one pound of beneficial microbes in our **gastrointestinal (GI) tract** and our skin is covered with bacteria (this is why elaborate washing procedures are required before surgery to remove skin bacteria that would otherwise get into the wound created by the surgeon). More importantly, this one pound of beneficial microbes in our **GI tract** produces beneficial compounds like **indole** derived from the amino acid tryptophan, which influences mood, brain development, aging, and liver function, as well as preventing invasion of the epithelial lining of the GI tract by pathogens.

To get to the lower regions of the GI tract, microbes must survive the acidic environment of the stomach (pH 1), as well as the membrane-dissolving bile acids that we secrete. To survive this rough passage through the upper GI tract, it is thought that microbes become dormant, so the low pH and bile acids do not kill them.

5. ***Microbes can be practically immortal, living for over 100 million years!*** Recently, bacteria as old as 101.5 million years were harvested from the bottom of the sea at the abyssal plain of the South Pacific Gyre and found to be alive! Because the ocean is so still in this region, there are few nutrients to support life. In contrast, there is plenty of oxygen, and it accumulates since there is so little life to utilize it. The lack of food forces the bacteria to basically stop growing or to grow incredibly slowly. Remarkably, these bacteria are not primarily spore-producers but are non-sporulating aerobes. Even more remarkable, when presented with food, most of the bacteria began growing immediately, with some doubling every 6 days, which is fast growth for a cell that has been asleep for 100 million years! So, in effect, these ancient bacteria have lived for over 101.5 million years, which makes them immortal. Moreover, unlike the fossil remains of dinosaurs, which are clearly dead, microbial dinosaurs are alive and grow today as they did over 100 million years ago. Therefore, when grow them today, we gain insights into how life played out over 100 million of years ago.

6. ***Dormant microbes may arise and reconstitute infections.*** For medical applications like treating microbial infections of the body, such as those of the ear, wounds, and stomach (e.g., ulcers), it is important to realize that most of these infections occur via pathogens making use of **biofilms** to protect them. Unfortunately, most of our medicines (i.e., antimicrobials like **antibiotics**) have been focused on killing actively-growing microbes, but many microbes in biofilms are not actively-growing. This often leaves the dormant cells unaffected. Therefore, after the course of antibiotics is finished, the sleeping and unharmed pathogens may wake to restart the infection. Hence, it is important now to be able to detect these unharmed, sleeping microbes and to develop pharmaceuticals that eliminate both growing and non-growing cells. Fortunately, compounds like mitomycin C can enter and destroy dormant bacteria so both growing and non-growing pathogens are removed.

Relevance for Sustainable Development Goals and Grand Challenges

- **Goal 1. End hunger, achieve food security and improved nutrition and promote sustainable agriculture** (*end hunger and malnutrition, increase agricultural productivity*). Farm animals for human food production have a negative impact on the environment. For example, the methane emissions from cows leads to global warming. In contrast, the use of microbes as a food source (especially protein) is perhaps a more efficient and more direct method to produce food.

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- **Goal 2. Ensure healthy lives and promote well-being for all at all ages** (*improve health, reduce preventable disease and premature deaths*). Farm animals are also frequently given antibiotics to increase their size. However, because they are mammals, they also have GI tract bacteria, and these bacteria that are exposed to the antibiotics and may become resistant to them. Once resistance occurs in the bacteria associated with farm animals, it may spread to areas that affect humans and renders current antibiotics less effective for human disease. Another important aspect of our unique GI tract bacteria is that each of us may respond differently to the same pharmaceutical; hence, knowledge of our gut microbiome may be important for understanding how we respond to medicine.

Potential Implications for Decisions

1. *Individual*

a. If alcohol kills bacteria on surfaces, what does alcohol do to the one pound of bacteria in your GI tract?

b. If antibiotics kill good and bad bacteria, what is the effect of taking oral antibiotics on the one pound of bacteria in your GI tract?

2. *Community Policies*

a. If bacteria can exist in a sleeping state and most antibiotics/antimicrobials/disinfectants kill only growing bacterial but have little effect on sleeping bacteria, what is the effect of using these compounds that are not completely effective during food preparation for the grocery store and for restaurants if not all disease-causing bacteria are killed?

3. *National Policies*

a. How can the safety of our food supply be improved? Emphasize the need to look for sleeping bacteria as well as active ones in food for sale.

Pupil Participation

1. *Class discussion of the issues associated with microbial viability*

a. **Biofilms:** Ask the class if they have ever picked up a rock from a river and what did it feel like? Explain the slimy layer is a biofilm of microbes cemented to each other and the rock. Explain this is what a dental hygienist removes from our teeth.

b. **Persistence:** Ask the class if they have ever surprised a turtle. Explain a turtle withdrawn into its shell is like a persister (dormant) microbe. Like the turtle once it is over the surprise (or after the dog leaves), persists wake up when food is presented and grow.

c. **Dead microbes:** Ask the class if they have ever picked up a seashell on a beach. If there is nothing inside, then this is the way dead microbes are.

d. **Microbes in our GI tracts:** Ask the class if they ever pooped. If so, then explain, ½ of what comes out are bacteria and the other ½ are the things our bodies could not digest fully.

e. **Microbes as food.** Discuss the possibility of utilizing rapidly-growing microbes as a source of food to replace animals. Also, discuss the need to detect dormant bacteria in food to prevent food poisoning.

f. **Antibiotic resistance and personalized medicine.** Discuss the need to discontinue the use of antibiotics on farms and on humans with viral infections. Also, discuss the importance of personalized medicine given our unique GI tract bacteria.

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2. *Pupil stakeholder awareness*

- a. Antibiotics are great for curing infections. Ask the class if they can see the negative effects, too (like killing the beneficial microbes in our GI tract).
- b. If each of us is unique in regard to the different microbes we have in our GI tract, ask the class how medicine can be improved by taking into account this uniqueness.

3. *Exercises*

- a. Calculate how long it would take for a single microbe to reach the mass of the Earth if it doubles every 20 min.

The Evidence Base, Further Reading and Teaching Aids

- Hans-Curt Flemming and Stefan Wuertz, “Bacteria and archaea on Earth and their abundance in biofilms,” *Nature Reviews Microbiology* 17: 247–260 (2019).
- S. Song and T. K. Wood, “‘Viable but non-culturable cells’ are dead,” *Environmental Microbiology* 23: 2335–2338 (2021).

Glossary

- ATP:** adenosine triphosphate, energy source that powers enzymes in cells, like a battery for a toy.
- Antibiotics:** compounds that stop the growth of microbes.
- Biofilms:** sticky microbial homes in which the cell is cemented to a solid surface and to other cells. Frequently called slime.
- ‘cell phones’:** chemicals secreted by microbes that allow them to communicate to each other.
- Communities:** groups of similar and non-similar bacteria in a biofilm, like you and your brothers and sisters and other children in day care.
- Dead cells:** cells that no longer can reproduce in the presence of food, usually indicated by a compromised cell membrane or hollow structure.
- Disassembly:** taking apart a biofilm, like taking apart a puzzle.
- Dormant:** sleeping microbes, like children at night.
- Exponential growth:** microbes usually split into two then the two cells become four cells, then the four cells become eight, etc. For example, your e-mail or problems during a pandemic.
- Indole:** secreted chemical signal derived from the amino acid tryptophan.
- Gastrointestinal tract:** your mouth to bottom digestive tract.
- Live cells:** cells which can grow in the presence of nutrients.
- Persistence:** state in which microbes are sleeping, like you at night, if you did not get taller when you sleep.
- Quorum sensing signals:** chemicals secreted by microbes that allow them to communicate to each other.
- Spores:** resting state in which microbes form a hard, resilient shell to withstand harsh temperatures and lack of food. For example, like a turtle withdrawing into its shell.
- Subsurfaces:** surfaces below the ground on which we walk.
- Suspended animation:** stopping something from growing by putting it in a very cold freezer.