

Environmental Chemistry and Microbiology
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Module - 7
Lecture - 35
Overview of Microbial Life - III

Welcome, everyone. This is lecture 5 (lecture 35) and the last part of an overview of microbial life.

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CONCEPTS COVERED

- Lecture 33**
 - Classification of living organisms
 - Prokaryotes and eukaryotes: cell structure
- Lecture 34**
 - Microbial Groups
- Lecture 35**
 - Prokaryotic diversity
 - The Microbial Genome

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As I mentioned in the previous slide, we have already covered the classification of living organisms; prokaryotes and eukaryotes; and different microbial groups. In this particular lecture, we are going to be looking at prokaryotic diversity and the microbial genome.

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Factor	Descriptive Term	Limits	Habitat	Domain	Example
High Temperature	Hyperthermophile	106 – 113 °C	Submarine hydrothermal vents	Archaea	<i>Pyrolobus fumarii</i>
Low Temperature	Psychrophile	-20 – -25 °C	Ice	Bacteria	<i>Synechococcus lividus</i>
Low pH	Acidophile	-0.06 - 4	Volcanic springs	Archaea	<i>Picrophilus oshimae</i>
High pH	Alkaliphile	8.5 - 12	Soda lakes	Archaea	<i>Natronobacterium gregoryi</i>
Pressure	Barophile	500 – >1000 atm	Mariana Trench	Archaea	Mariana Trench MT41
Salt (NaCl)	Halophile	15% - 32%	Salterns	Archaea	<i>Halobacterium salinarum</i>

Extremophiles

Brock, 2003



So, I think I mentioned at some point that we have 3 domains; we have bacteria, we have archaea, and eukarya. Now, eukarya means all eukaryotes. And all prokaryotes are divided into 2 domains, bacteria, and archaea. So, I also mentioned I think at some point that archaea are bacteria that perhaps started life in an environment that is nothing like what we see today. So, remember that life did not begin under conditions that we see today.

Life began under very, very harsh conditions. The temperature was very high; pH was somewhat low; there was no oxygen in those conditions; those are the conditions under which life began. So, these archaeobacteria are considered to be extremophiles. And that is what we are going to look at in this particular slide. So, here we have a few examples of archaeobacteria and just one example of a more modern bacteria or eubacteria or just bacteria.

So, let us take the idea of temperature first. We will be looking at these factors in more detail in subsequent topics. But this is just to give you a little bit of an idea about extremophiles. These are a very interesting group of bacteria. So, that is why it is important to at least recognize the fact that they exist. And they exist in very harsh conditions, which we would normally imagine, no life can exist.

So, at very high temperatures, we have a group of bacteria called hyperthermophilic bacteria or hyperthermophiles. They are capable of living within a temperature range of 106 to 113° C. Their habitat can be hot water springs or boiling water springs. They can be in submarine hydrothermal vents. So, you have hot gases coming out from the depth of the earth; in these marine environments, at the depth of the ocean floor, and so on. These are archaeobacteria. So, this is one particular example called *Pyrolobus fumarii*. At the other end of the temperature range, we have what is called psychrophilic bacteria. These psychrophilic bacteria are capable of thriving under sub-zero conditions. So, we have one particular species *Synechococcus*

lividus, which is capable of living even in ice. So, people have bored into Antarctic ice and found examples of algal as well as bacterial cells that have been able to thrive even under those conditions.

Then we have low pH. Those are called acidophilic bacteria. In this particular example, it is a volcanic spring, but you can see acidophilic bacteria in acid mine drainage. The limits in terms of pH are negative units of 0.06, all the way to pH 4. And these are again archaeobacteria. And in this particular example, it is *Picrophilus oshimae*. And high pH which means base. They are thriving in a high pH environment.

So, it is an alkaliphile or an alkaliphile, 8.5 to 12 pH. Soda lakes are examples of where you might find these bacteria. And these are again archaeobacteria. At the bottom of the ocean or in the depths of the oceans where the pressure because of the hydrostatic pressure is extremely high, there are certain living, there are very few living organisms that can withstand those kinds of hydrostatic pressures. So, the number of organisms you see in the depth of the oceans is much less than what you see at the higher layers. So, these barophilic bacteria are capable of withstanding pressures of 500 to more than 1,000 atmospheres. And the Marianas Trench which is considered to be the deepest point on the planet, some bacteria have been found even in that location. It is again an archaeobacterium. And it has been named for the location, so, it is called Mariana Trench MT41.

You would think that salt which we think of as a preservative; when we think about food preservation, we think of high saline solutions as being preservatives because most bacteria or pathogenic bacteria are not capable of growing in them. So, that is definitely true. However, that does not mean there are no bacteria in the salt pans. If you happen to go to a salt pan, you might want to take a sample and see if there are bacteria in it, because halophilic bacteria are capable of thriving even in those environments. So, they can tolerate saline concentrations of 15 to 32%. What is the saline concentration of seawater? Seawater is 3.5%. So, this is almost 10 times higher than seawater. So, even under those conditions, there are certain algal cells as well as bacteria that are capable of growing and surviving. So, this is an example of archaeobacteria. It is a *Halobacterium salinarum*. And I will show you some graphics, which also give you examples of the same.

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Prismatic Spring, Yellowstone National Park. The breathtaking colors are attributed to the various species of thermophilic bacteria living in the spring. The blue water in the center is very hot, but it may support chemotrophic life – a chemotroph is an organism that uses chemicals for a source of energy.
 Cred: By Jim Peaco, National Park Service - <https://commons.wikimedia.org/w/index.php?curid=326389>

Pyrococcus furiosus. A hyperthermophile archaeon. It grows best at 100°C.
 Cred: By Fabio314 - Own work, CC BY-SA 3.0, <https://commons.wikimedia.org/w/index.php?curid=26052758>

Extremophiles

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So, here we have a boiling water spring. This is from the Yellowstone National Park. All the colors are due to different species of thermophilic bacteria that live in this particular spring. The blue water in the center is extremely hot. It is boiling, but it does not prevent particular species of bacteria from growing in it. Here is another example of hyperthermophilic archaeobacteria. The optimum temperature for its growth is 100°C. So, this is *Pyrococcus furiosus*.

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Freeze-etched
 0.5 µm

Aquifex. This hyperthermophile bacteria are the 'early-branching' species of 'Bacteria'. They grow optimally at >80 °C.

Extremophiles

Deinococcus radiodurans is listed as the world's toughest bacterium in the Guinness Book of World Records as it is the most radiation resistant organism known. It can survive at extreme low temperature and pH, without water, and air (in vacuum).
 By Credit: TEM of *D. radiodurans* acquired in the laboratory of Michael Daly, Uniformed Services University, Bethesda, MD, USA. <https://commons.wikimedia.org/w/index.php?curid=157172>

Phylogenetic tree based on Woese et al. rRNA analysis.
 Cred: By Maulucioni - Own work, CC BY-SA 3.0, <https://commons.wikimedia.org/w/index.php?curid=24740337>

Brock, 2003

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So, here we have hyperthermophilic bacteria. This is a SEM. This is a Scanning Electron Micrograph. You can see it. And you can see the size; it is a very small-sized bacteria. This is an *Aquifex* bacteria that has an optimum temperature of growth, which is greater than 80°C. You can see the tree of life; just to remind you of the phylogenetic tree. And this is the last universal common ancestor. And you have the 2 branches with the prokaryotes bacteria and

archaea; and eukarya which is the third branch or the third domain. Then we have another extremophile called *Deinococcus radiodurans*. And this is considered the world's toughest bacterium, according to the Guinness Book of World Records. This bacterium can withstand radiation that no human being or any other organism is capable of withstanding.

It can survive under extremely low-temperature conditions and pH. It can live without water, without air, and so on. So, these are examples of extreme thermophiles or extreme extremophiles.

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Colonies of *Halobacterium salinarum* growing on salt-saturated agar plate. It is found in salted fish, hides, hypersaline lakes, and salterns. Cells of *Halobacterium* can live within the salt crystals.
 Credit: https://microbiologyociety.org/resource_library/knowledge-search/the-immortal-halophilic-superhero-halobacterium-salinarum-a-long-lived-poly-extremophile.html

South San Francisco Bay, California. The red color in these ponds is from *Dunaliella salina*, a micro-algae containing high amounts of beta-carotene. *Dunaliella salina* is halophile.
 Credit: By Doc Searls from Santa Barbara, USA - 2009_11_26_bos-8fo_2733/loaded by PDTilman, CC BY-SA 2.0, <https://commons.wikimedia.org/w/index.php?curid=9049595>

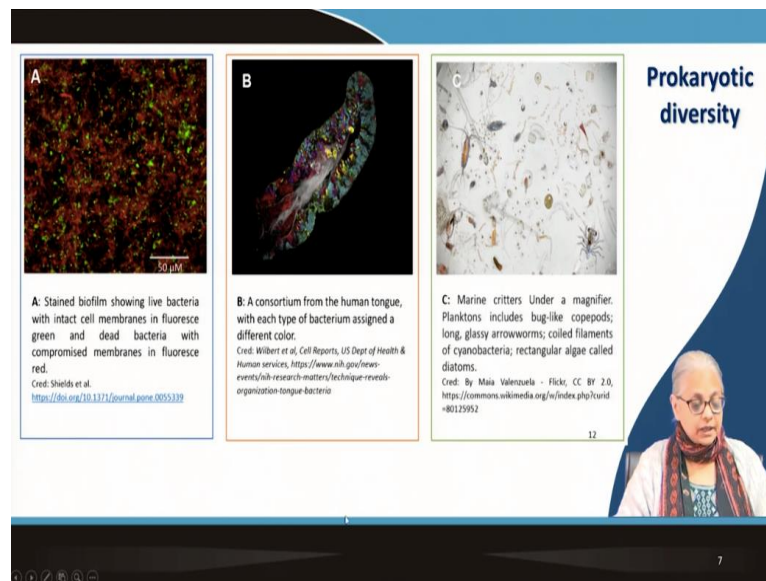
Thermococcus gammatolerans are extremophiles and are radiation resistant, up to 30,000 Gy gamma rays. A dose of 5 Gy is sufficient to kill a human.
 Credit: By Angela Tapias - Archivo Angels Confalonieri, CC BY 3.0, <https://commons.wikimedia.org/w/index.php?curid=381702>

Then, as I said, *Halobacter salinarum*. This is an example of a bacteria that is capable of growing in salt pans. Remember, the concentration of salt in salt pans is much higher than in seawater. So, even in marine seawater, you will find that very few bacteria are capable of surviving. So, some species are capable of surviving in these harsh environments.

This particular graphic is of a bacteria growing on salt-saturated agar plates. You can find them in salted fish; you can find them in hides, animal hides; hypersaline lakes, and salt pans and salterns. This graphic shows you algal growth in ponds. So, this is a red algae, *Dunaliella salina*, which has very high amounts of beta-carotene. And it is a halophile. So, it is red in color. In fact, I should mention one more point here, that sometimes you can see a pinkish color where you have high saline solutions or high saline-containing wastewaters. You will find this pinkish color. And that pinkish color may be due to either the bacteria that are growing in these conditions or due to these red algal cells. So, it depends, you have to test the sample for all these possibilities.

Then you have, like I said, radiation-resistant extremophiles, *Thermococcus gammatolerance*. This is an extremophile. It is extremely resistant to radiation. It can withstand 30,000 grays in terms of gamma radiation. And for comparison, 5 grays are sufficient for killing a human being. So, you can see that these bacteria; are all archaeobacteria. And these archaeobacteria go back to a time when the conditions on the planet were extremely hostile compared to current conditions, but they are reminders of that past.

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I have already explained this slide, but just to remind you that there is a lot of prokaryotic diversity in so many different environments. So, this is what you might find in a wastewater sample. This is what you find on the surface of the human tongue. This is what you can see in marine environments. You take a sample from a pond, freshwater pond. You will again find it teeming with life. You will find algae, bacteria, protozoa; you name it. All these microorganisms show up under the microscope.

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Prokaryotic diversity

Gram positive bacteria *Streptomyces* are filamentous and are found in soil and mud. They are known to exude the chemical 'geosmin' which is responsible for the earthy smell of wet soil. Animals and humans are sensitive to the smell of geosmin. It helped our ancestors to find water.

Gram negative bacteria like *Pseudomonas aeruginosa* do not retain the crystal violet stain used in the gram-staining method of bacterial differentiation. *Pseudomonas aeruginosa* is infectious to humans and some animals. *Escherichia coli* also known as *E. coli* is another example of a gram negative bacteria.

Pic cred: Public Domain, <https://commons.wikimedia.org/w/index.php?curid=716469>

Cred: By Y. tambe - Y. tambe's file, CC BY-SA 3.0, <https://commons.wikimedia.org/w/index.php?curid=49535>

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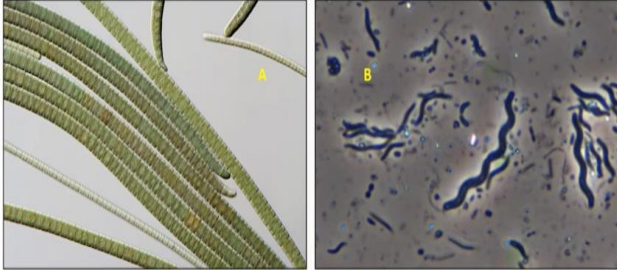
Then you have *Streptomyces*. So, this is a gram-positive bacterium, which is filamentous in nature. You can see how individual cells have formed these long filaments. They almost look like the fungi that we were looking at, and that is why the name *Streptomyces*. It is filamentous. It is found in soil and mud. And most of us have experienced this earthy smell. So, when you have dry soil becoming wet, there is a release of a particular compound that has this beautiful earthy smell that most of us like, and that is due to a particular compound called geosmin. And animals and human beings are very sensitive to the smell of geosmin because that was what helped us find water. So, not only do we find it extremely pleasant, but it is also an indicator of the presence of water.

Then we have Gram-negative bacterium like *Pseudomonas aeruginosa*. Now, *Pseudomonas aeruginosa*, because it is Gram-negative, it means it cannot retain the crystal violet stain. So, we will talk about the Gram staining procedure in the microscopy topic. But for now, it is just simple; it is enough for me to say that for reasons of the structure of the cell wall, gram-negative bacteria are not going to retain the first stain which is the crystal violet stain, and the second stain which is the counterstain safranin gives it an orange color. So, what you see over here is an orange color.

That is because the bacteria *Pseudomonas aeruginosa* is Gram-negative. *Pseudomonas aeruginosa* is all around us. It is present in the soil; it is present in the feces of human beings, animals, perhaps even birds. It is similar to *E. coli*. *E. coli* is also another one that is found in the feces of humans and animals and birds and so on. So, these are both gram-negative bacteria that are very common in our environment. You will find it in water, soil, all of these environments; not in air; they need water.

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Prokaryotic diversity



Filamentous cyanobacteria A) *Oscillatoria* and B) *Spirillum*. Cyanobacteria created the oxygen in the atmosphere today through photosynthesis. They are found in many other morphological forms; unicellular, colonial, and heterocystous. Heterocysts carry out nitrogen fixation.


Credits: A) By Wiedehopf20 - Own work, CC BY-SA 4.0, <https://commons.wikimedia.org/w/index.php?curid=45240367>
 B) By Wolfram Adlassnig - Own work, Public Domain, <https://commons.wikimedia.org/w/index.php?curid=3192456>

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Then we come to filamentous cyanobacteria. So, we have 2 examples here, *Oscillatoria* and *Spirillum*. So, cyanobacteria are - there was a point in time when they used to be called blue-green algae. But cyanobacteria are considered to be the ones that have modified the planet's atmosphere from anoxic conditions to the present oxic conditions. So, the oxygen in the atmosphere that we see today, is a fairly high amount of oxygen, 29% (correction - 21%). And that amount of oxygen has been created by these cyanobacteria. Without them, none of us would be here. And they are also found in several different forms. They are found in unicellular forms, colonial forms, heterocystous forms. The heterocystous forms are the ones that are capable of fixing nitrogen from the atmosphere. Remember that nitrogen gas that is present in the atmosphere; is a huge amount, but it is not bioavailable. And unless you have bioavailable nitrogen, you cannot have productivity. Even plants, photosynthetic organisms need bioavailable nitrogen to survive and grow. So, all life on the planet literally depends on the presence and availability of bioavailable nitrogen. So, that too is attributed to these cyanobacteria.

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The microbial genome



- All genetic material = genome = the entire DNA
- Gene = segment of DNA that encodes a protein (via messenger RNA) or another RNA molecule like ribosomal RNA
- Prokaryotes have a large, single, generally circular, double-stranded DNA molecule = chromosome; nucleoid
- Eukaryotes have linear DNA molecules in the nucleus, packaged in an organized state called chromosomes
 - Two copies – diploid; Single copy - haploid
 - Cell reproduction = doubling of chromosomes and then cell division (mitosis)
 - Sexual reproduction = diploid genetic material is halved in meiosis
 - Baker's yeast, *S cerevisiae* contains 16 chromosomes – 8 pairs
 - Human cells = 46 chromosomes, 23 pairs



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We now come to another interesting aspect about microorganisms, and that is the genetic material. So, when we talk about the genetic material, the entirety of the genetic material of an organism is called its genome. So, you have heard about the Human Genome Project. So, here we are going to restrict ourselves to looking at the microbial genome and how does it compare to the genome of other organisms.

So, the entire DNA which is the ge-nome or the gen-ome, whatever you want to call it; is the entirety of the genetic material or the DNA. Here we have the entire DNA. And now this DNA has different segments on it. And you will find that if you look at any particular chromosome, you will find that there are segments that have information that is utilized by the organism to code for a particular protein.

So, let me try and draw this. OK, so this is a very rough sketch of what a particular chromosome might look like. So, you have a long strand of the DNA. And you will have segments. And each segment that encodes for one product or one protein is called a gene. So, every segment that encodes for one product, by definition is called the gene. You will have strands that do not encode for anything. So, they are called that nonsense code. And they are not equal in size. You might have small genes and large ones and so on. So, that is, each gene is a segment of the DNA that encodes for one product and that product is generally a protein. And this can be encoding. The encoding; remember, I said in a previous lecture that the DNA is transcribed to the RNA, and then the RNA is translated to a protein. So, those are the 2 processes that have to happen for the proteins to be formed. So, this can happen via the messenger RNA; or it can happen through another RNA or something like the ribosomal RNA.

Now, for the prokaryotes. Since we are going to be focusing more or less on prokaryotes, mostly on bacteria, and we know that these prokaryotes have a large, single, circular, double-stranded DNA; that is one single chromosome. So, these prokaryotes do not have more than one chromosome. They have just one chromosome and it is a single circular chromosome. And they do not have a well-defined nucleus. This single chromosome is scattered throughout the cytoplasm of these prokaryotes.

And so, eukaryotes have linear DNA in the nucleus. And because the nucleus is very well-defined, the chromosomes, whether they are a single chromosome or multiple chromosomes, they are packaged in a very organized state.

Now, if there are 2 copies of the chromosome, it is called a diploid cell. If there is a single copy, it is called a haploid cell. For cell reproduction to happen, these chromosomes have to be doubled; and then the cell divides. So, for the new biomass to be generated, you need doubling of the chromosomes followed by cell division. In sexual reproduction, the diploid genetic material is first to split into 2 haploid cells in a process called meiosis.

Cell division is mitosis, and the halving of the diploid cell is always; it happens only in sexual reproduction, and that happens by the process of meiosis. Then you have yeast (correction: not bacterial). Just an example, *Saccharomyces cerevisiae*, which has 16 chromosomes, which means 8 pairs. The human cells have 46 chromosomes, which means 23 pairs.

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Nucleus or nuclear region

- Genome → one or more chromosomes: each distinct DNA molecule → multiple genes: DNA segments on the chromosome with coding for specific single products: 1 product → 1 gene → Millions of nucleotide base pairs (Mbp) → MW (500 x bp)
- Bacteria have a single chromosome with 3000 to 4000 genes (See Figure 4.8, Brock, 2015)
- Humans have 23 pairs of chromosomes (22 autosomes and 2 distinct sex chromosomes) with the largest having 2968 genes and the smallest Y chromosome having the fewest genes (231)
- While most prokaryotes have a single circular strand of DNA, bacteria with linear DNA have been observed
- Eucaryotes have linear DNA

<https://www.news-medical.net/life-sciences/DNA-Biological-Functions.aspx>

Normal Human Karyotype

Autosomes

Sex Chromosomes

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So, this is a graphic. On the upper right, you can see a graphic that shows the 23 pairs of chromosomes that human beings have. And out of these 23, 22 are autosomes and there is only one pair of sex chromosomes; that is the ones that differ, define the gender. So, XX represents

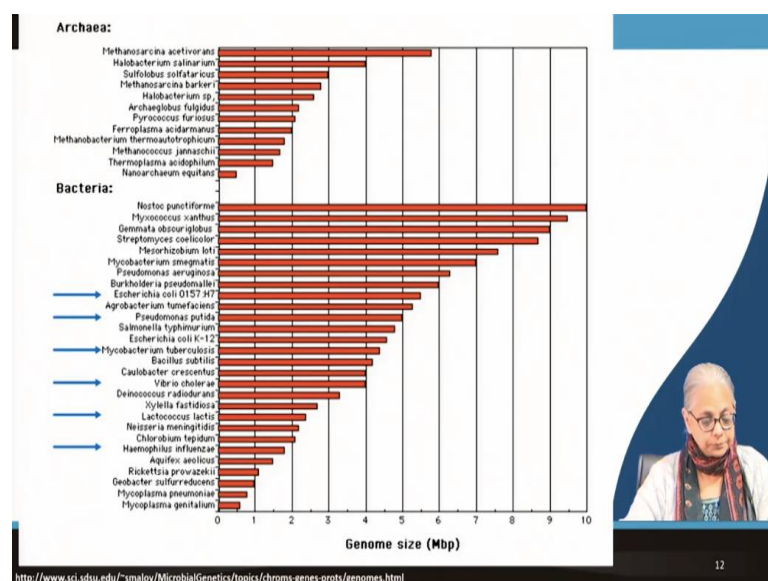
females and XY represents males. Coming back to the genome and the terms that we use to describe the genetic code.

So, the genome means the entirety of the DNA. It has one or more chromosomes. So, bacteria have only one chromosome. Higher organisms will have more than one chromosome. And each DNA molecule will have segments. I have already shown you that. So, every single chromosome has several genes. It is not just one gene; every chromosome has several genes. These DNA segments on the chromosome are encoding for separate products.

So, by definition, a gene is that segment of the DNA that encodes for a particular product. And a gene can have millions of nucleotide base pairs, or it may have a few 100 or a few 1,000 base pairs. So, that is the correspondence between gene to product to base pairs. I have already mentioned that bacteria have only one chromosome with about 3,000 to 4,000 genes. And we know that human beings have 23 pairs of chromosomes, with the largest one having 2968 genes and the smallest one, the Y chromosome has the fewest number of genes; and that is 231 genes. These prokaryotes have a single circular strand. So, if you were to refer to one of the textbooks or even both the textbooks, you might find some graphics that show you the single circular strand. It is a very long strand. It is not just a very short strand. It is an extremely long strand, which has a lot of information encoded in it, but it is a single circular strand. So, we can represent it by a simple circle.

So, that is our single chromosome in a prokaryote. On the other hand, eukaryotes all have linear DNA. They do not have circular chromosomes. There are a few exceptions in the literature, where people have reported bacteria with linear DNA. By and large, all prokaryotes have circular stranded DNA.

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So, here we have; in this particular graph, we have a comparison of the genome sizes of archaeobacteria and bacteria. Now, as I said, archaeobacteria go back to a much more primitive form of life. And you can see that the genome size on average is much smaller for archaeobacteria compared to bacteria. So, you can see all these extremophiles that we were looking at earlier.

The methanogenic bacteria; halobacteria that grow in salt pans; hyperthermophilic bacteria; all these bacteria have a size range anywhere from 0.5 million base pairs to about a little less than 6 million base pairs. In comparison, if you look at the more modern bacteria, you can see, they range from almost the same minimum, 0.5 million base pairs; but the largest ones are close to 10 million base pairs.

And the ones that we are most familiar with within our environment; *E. coli*, which is one of them; 5.5. It is close to the largest archaeobacteria. *Pseudomonas*, which is another very common species found in our environment, *Pseudomonas putida*; *Mycobacterium tuberculosis*; all of these are fairly large, in general, compared to archaeobacteria.

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Organism	Genome size (bp)*	Estimated genes
Human (<i>Homo sapiens</i>)	3.1 billion	30,000 to 40,000
Laboratory mouse (<i>M. musculus</i>)	1.3 billion	30,000
Mustard weed (<i>A. thaliana</i>)	60 million	25,000
Roundworm (<i>C. elegans</i>)	48.5 million	19,000
Fruit fly (<i>D. melanogaster</i>)	70 million	13,000
Yeast (<i>Saccharomyces cerevisiae</i>)	12.1 million	6,000
Bacterium (<i>E. coli</i>)	4.6 million	3,200
Human Immunodeficiency virus (HIV)	9400 bases	9
Corona virus (single stranded RNA)	29811 bases#	

#Nature Education, *Wikipedia

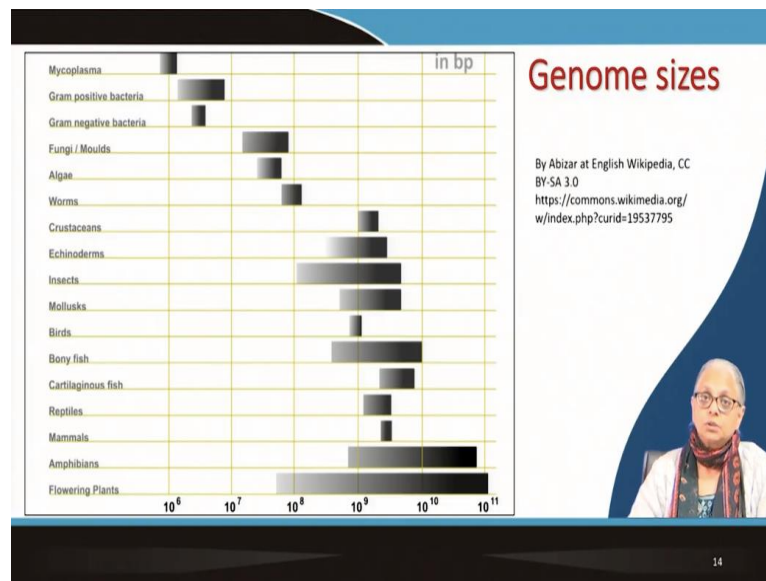
Now that we have seen the genome sizes of archaeobacteria as well as bacteria, let us get some idea in terms of other organisms. So, in this table, there are genome sizes for organisms starting from the Coronavirus to human beings. So, when we look at human beings, we have a genome size of 3.1 billion base pairs; and the estimated number of genes is 30,000 to 40,000.

A lab mouse would have 1.3 billion base pairs and genes would be around 30,000. Mustard weed has about 60 million base pairs and 25,000 genes. Roundworms have about 48.5 million

base pairs and 19,000 genes. So, the fruit fly has about 70 million base pairs. Yeast, which is a microorganism has 12.1 million base pairs and 6,000 genes. *E. coli*, which is a bacteria has 4.6 million base pairs and 3,200 genes.

Let us compare that with 2 retroviruses, the Human Immunodeficiency Virus, HIV, and the Coronavirus. The human deficiency virus, the HIV virus has 9,400 bases; not base pairs but bases and 9 genes. Coronavirus, which is a single-stranded RNA; that is why it cannot be expressed as base pairs; it has to be expressed only in terms of bases; has 29,811 bases. This information was from Nature, the magazine.

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So, in this slide, what you see is more of the same comparison but this is shown in graphic form. So, you can see different types of Gram-positive and Gram-negative bacteria, in terms of base pairs. The size of the genome is anywhere from 10⁶ to 10⁷ base pairs and fungi. Fungi range from 10⁷ to 10⁸. So do algal cells and so do some worms.

Crustaceans, echinoderms, insects; all of them range over a fairly large size, from 10⁸ to 10⁹ or even 10¹⁰. And birds are around 10⁹. Fish are around 10⁹ to 10¹⁰. Reptiles, again in the same range. Mammals, within that range. Amphibians, flowering plants, all have a huge range from 10⁸ to 10¹¹ base pairs.

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We will stop at this point. Thank you.