Microbiology Sheet # 4

last lecture we mentioned the technique which is most used in laboratories to get a pure bacterial culture which is the **streak plate** technique.
With each streak, we dilute the original bacterial sample in the agar, and after 4-5 streaks, we end up with few bacterial colonies which are far away from each other and are identical, and they represent majorly the bacteria which is found in the highest concentrations in the original sample "the pathogenic bacteria".

Another techniques for isolating pure bacterial cultures are the pour plate and the membrane filter techniques.
In **pour plate**, we take a sample from an infection and we dilute it, this way we insure that the majority of the bacteria in the solution is the pathogenic bacteria, then we mix this solution with the agar before its poured into the petri-dish and incubate them.
After being incubated, we will observe bacterial colonies, Which might be either above the agar or inside it. The ones outside the agar are aerobic bacteria and the ones within are anaerobic (since no oxygen).

In **membrane filter** technique, the sample is also diluted but is collected on a membrane (like a filter paper). The water moves though the membrane and the bacteria is held on top. Afterwards, this membrane is placed upside down on the agar and it incubated to get distinct colonies.
This technique is mainly used in testing water contamination with sewage/fecal material. (If E.coli is present, then its contaminated. Since E.coli grows in the intestine of human beings).

Afterwards we take a sample from these few "pure" colonies, to identify the bacterial type.

How to identify the bacteria?

From morphology:

1. looking at the morphology of the colonies themselves might give you an indication (shape/size/color/ texture)
2. take a sample, put it on a slide
3. stain it to identify weather gram +ve or gram -ve
4. observe under the microscope to know the shape of the bacteria (coccus/bacillus/coccobacillus...etc)

By chemical means:
- culturing the bacteria again and place it in a selective media or an indicator media to know its type

By serology:

-Serology is the use of antibodies.
By making antibodies against certain antigens on bacterial surface, these antibodies-which are specific to a certain bacteria- can be used to identify the presence of this specific bacteria by attaching to them in the sample.
These antibodies are labeled by florescence to be identified.
This is mainly used to identify the genus and the species of the bacteria.
If the antibodies were made against the O-polysaccharide, this is to identify between different strains of the same bacterial species.

*After identifying the type of bacteria, we determine its sensitivity to antibiotics (we will talk about it later)

Bacterial Growth Phases

1**.** Lag phase: there are no increase in numbers, only in size and mass of the bacteria (they are preparing to divide)

2. Exponential phase: increase in numbers exponentially in very short time. The exponential phase doesn't go on forever due to limiting factors (ex: nutrition) or toxin build up or running out of space...etc

3. stationary phase: when the exponential phase stops due to the reasons mentioned above, the stationary phase starts, were there's no replication or growth of bacteria (their number stays constant and they are still alive)

4. Death phase: a depth in the growth phase occurs to represent the dying bacteria. The number of actual bacteria stays constant (alive + dead bacteria), only the number of **alive** bacteria drops.

**The constant numbers are indicated by the turbidity (optical density) of the culture (the dotted line in the curve).
The more cells (bacteria) in the solution, the more the turbidity. Therefore the line goes up in the exponential phase, and stays constant in the stationary and death phases (constant number of bacteria)

Method of measurement of cell mass

There are direct methods (Physical measurements and Chemical measurements) Or indirect methods (measurement of chemical activity). These are mainly used for research purposes.

Turbidity measurements are the ones most commonly used in labs and not only for research.
These are done by shining a light through a suspension containing the bacteria and the brightness of light transmitted to the other side is measured. The more the bacteria in the sample the less the transmitted light and the more the turbidity (optical density).

Methods for measurement of cell numbers

1. direct counts (visually: by counting chambers under microscope
or electronically: by laser beam)

2. indirect viable cell counts.
This is used when we are interested in counting the number of only alive bacteria. The sample is spread on agar, and only alive bacteria would form a colony (colony forming unit-cfu), then the number of colonies is counted representing the number of bacteria's in the sample (since each bacteria forms a colony).
But this isn't practical to be used in case of bigger samples with a huge number of bacteria. Therefore, the sample is diluted by a method known as **serial dilution**, to decrease the number of bacteria and to be able to count them more easily.

How is serial dilution done?

If we have solution of approximately 50.000 bacteria/mL, 1 mL is taken from this solution and diluted in 9mL of water in a dilution ratio of 1:10. This would result in a solution with 5000 bacteria/mL (dilution factor = 10)
Another 1 mL is taken from this solution and diluted in 9mL of water (1:100 dilution) resulting in a solution with 500 bacteria/mL (dilution factor = 100) and so on... until we reach a solution of 5 bacteria/mL. (If this is cultured we get 5 colonies)

The final dilute of let's say with dilution factor 100,000, is then the one that undergoes the indirect viable cell count. And the number of colonies formed after incubation is counted.

If the number of bacterias is unknown, we multiply the number of colonies (which represents the number of bacteria in diluted form) by the dilution factor (100,000) to get the original bacterial count in the original sample.

* note: we usually incubate more than one plate to count the average number of colonies for more accurate results.
*** generation time and growth rate:** NOT INCLUDED

Bacterial Genetics

-Bacteria has a single double stranded circular DNA, and some also have bits of DNA known as plasmids.
-Since bacteria undergoes rapid divisions, it's more likely to undergo mutations.
- A mutation is a change in the original nucleotide sequence in the DNA. (either by substitutions, deletions, insertions or rearrangements)
- A mutation can be either good (give a better protein) or bad (a non-functional protein)
- Transition of nucleotides is the substituting of purines by purines or pyrimidines by pyrimidines. Transversion of nucleotides is substituting a purine for a pyrimidine or vice versa.
- Any fault in the mismatch repair enzymes (that proof read and edit) would also increase the risk of mutations.
- Mutation in one bacteria is easily passed on to other bacteria's. And if this mutation makes the bacteria more pathogenic, this would lead to all other bacteria's to get this pathogenicity strain. Therefore, mutations in bacteria's can pose a problem.

Types of mutation

1. silent mutation: a change in a nucleotide sequence that would still give the same amino acid and the same final protein. (No harm)
2. Missense mutation: a change in nucleotide that would give another amino acid (final protein with one different amino acid). This change might or might not affect the final protein.
3. Nonsense mutation: a change in the nucleotide that would make the original codon that coded for a specific amino acid, a stop codon.
4. Frame-shift mutation: due to insertion or deletion of nucleotides not in multiples of 3.

5. Null mutation: a mutation in a gene which is not expressed.
6. Suppressor mutation: is a mutation that reverses a previous mutation to return the phenotype to its original normal form.
If this happens on the gene itself, it's called intragenic. If it happens outside the gene (after transcription or when the resulted protein is doing its function) then it's called extragenic.

* reminder: a codon: a sequence of 3 nucleotides that code for a single amino acid

Spontaneous mutations

Happens spontaneously without the interference of outside factors (mutagens).
Occurs due to electrochemical rearrangements.
For Example: tautomerization (changing of keto group (=0) in a nucleotide to an enol (-OH) would lead to abnormal hydrogen bonding between purines and pyrimidines.
EX: Thymine normally bonds to adenine, if it goes tautomerization, it bonds to guanine!

"ما المبدأ والأفكار بغير عقيدة حارة دافعة؟
إن المبادئ والأفكار في ذاتها -بلا عقيدة دافعة- هي مجرد كلمات خاوية أو على الأكثر معان ميتة.
والذي يمنحها الحياة هي حرارة الإيمان المشعة من قلب إنسان.
امن أولا أنت بفكرتك حتى الاعتقاد الحار, عندئذ فقط يؤمن بها الإخرون!
لا حياة لفكرة لم تتقمص روح إنسان ولم تصبح كائنا حيا دب على وجه الأرض في صورة بشر!"

- سيد قطب

Jumana Kussad