Microorganisms: Culturing And Isolation Techniques

Culturing and Isolation Techniques

Microorganisms are everywhere. “The word microorganism is origin from Greek. The very tiny thing, which have the presence of life and that tiny thing is only visible by using microscope means we cannot able to see that tiny organism with our open eyes. Microorganisms are as an example bacteria, fungi, protozoa, algae amoebas and slime molds. Peoples easily think that they effected by the microbes and this is the normal process. But they don’t know actually that they are the host of the microorganism, because the microorganism depend on them also sometimes they are beneficial for the human body”. (Microbe, 2019). Microorganism can develop in the natural habitat like land. They are staying with the others form of life. There are some microorganisms that create harm to the host and we actually called it pathogens. When they are enter out body and do some harm to the body, that time the body show some effect, it decided that how they actually affect with the patient body. “The separation and develop the microorganism in an unadulterated culture is very important to find out the microorganism and the treatment procedure. The culture we in nature, it is actually stay with the other bacteria, pathogens and microorganisms. But in the laboratory the individual species separated from one another. The culture, which has just one type of microorganism, that type of culture called pure culture. The process helps us to find out a pure culture by isolated one species of microorganism from the solution of other species, is known as separation of an organism. There are some special methods we are actually using to the purpose of pure culture of tiny organism. In some time the pure culture can get by straight separation or straight shifting. This technique can be applied on that situation where the pure cultivation of microorganism develops naturally. Types of sample of the microbes obtain from culturing will depend on the environment and characteristics of the microbes. Individual microorganism can be separated from body tissue and fluids like as blood, sputum, urine, pus, faces, spinal fluid, bile, pleural fluids, stomach fluids etc. If a patient suffering from typhoid fever, the bacteria Salmon Typhus may present in the blood stream. For obtain a pure culture, we are actually following different kind of method of isolation such as striking, plating, dilution, enriched method and single cell method”. (Obtaining Pure Culture of Microorganisms: 6 Methods, 2019). In this lab report I have included the streaking plate technique for isolating a microbes. The technique is broadly used in the procedure of separation of microbes. The method developed of pouring of an accurate safe sample in sterile petriplate and permit the medium to harden. By the lopping with the inoculating loops the one third of the diameter of the plate has been covered. This is the methods that isolated a pure sample of bacteria from the species of culture.

Aim: The aim of this experiment is to isolate specific single microbes from the various types of colonies of microorganism. Also see their shape, size, color and growth through this experiment.

Materials:

1. Sterile nutrient agar plate
2. Inoculating loop
3. Mixed culture in an environment
4. Bunsen burner

5. Gloves
6. Musk
7. Permanent marker
8. Incubator machine.
9. Hexisole
10. petri dish

Method:

1st Day of the experiment:

1. At the begging of the experiment, we have washed our hand properly with the soap and water.
2. Then we have worn the gloves and muck as a protective barrier.
3. After we have collected the sample from the refrigerator, which has been reserved to the refrigerator in a Petri dish.
4. The sample we have actually collected and the microorganism we have seen in the Petri dish, there are large in number of colony because of the multiplication of microorganisms.
5. Than we have light up the Bunsen burner.
6. Next we have taken an inoculating loop and gently put it on the fire of Bunsen burner and we wait till the color of the loop get red.
7. After some moment we have put out the inoculating loop from the fire and take some time so that the loop can cool down.
8. When the loop got cool we have gently put the loop on a single microorganism colony of the agar culture and by simply touched of the loop we take a very small amount of sample of microorganism.
9. Then we have placed the sample of microorganisms in the A section on the surface of new agar solution.
10. Then we gently streak the loop on the surface of agar solution. We draw some line from A to C section very smoothly by maintain some rules. We have draw one third of the plate
11. When the streaking has finished we have put the inoculating loop again on the fire Bunsen burner so that the microorganism on the loop get might dead.
12. After we have put the Inoculating loop on proper place.
13. Next we have put the agar culture in the incubator at the temperature of 37 degree Celsius.
14. We have put off the gloves and musk and discarded it in the dustbin. Next we have washed our hand properly and leave the lab.

2nd day and 3rd day:

1. We have maintained proper handwashing and protective barrier.
2. We have collected our culture from the incubator machine and observed the changes in our culture and note it on the notebook.

Result:

After 24 hour observation:

Figure 1: microorganism colony “Streaking plate culturing method”

1. There were 15 colonies of the bacteria except the line. Everyone is small size and round shape
2. A big irregular shape and big size of fungus colony visible in the middle and get touched with the margin of the bacteria. The color was off white.
3. In the streaking line we have found 44 small sizes and round shape bacteria colony in the C section. Each of them color was brown and in the joint of B & C section there were 14 colony of bacteria.
4. At the beginning of the A section, we have seen that there were a red bacteria colony was present , similar as the sample we have taken..

After 48 hour observation:

Figure 2: The picture of microorganism colonies after 48 hour

1. The fungus colony in the middle portion was big in size than previous (24 hour) and it was crossed and breaks the lines of bacteria colony.
2. There are also 3 round shapes and medium sizes whitish color funguses were present.
3. Except the streaking 23 external bacteria small and round shape colony were present in the culture. Among them 6 has brown color and 17 has white color.
4. In the c section we saw that the tail size of the bacteria colony got thin and now this time we are not able to count the number. Just 1 colony of the bacteria with round shape were present in the last portion of the tail and the colony color is yellow.
5. We also observes that after the 24 hours the color of the bacteria was slightly red but now this time the color of the line of bacteria colony fully red.
6. The number of the colonies of microorganisms was more and the size of each line segment was thin than 24 hours.

After 48 hour observation (environmental):

Figure: Streaking Plate microbes culturing (environmental)

After 48 hours we have found a brown type bacteria colony by the streaking method in an agar solution. In the culture, there were also other types of microorganisms is present as like Staphylococcus, streptococcus and fungus.

1. There were individual 15 bacteria colony in the plate & there size is small.
2. About 6 staphylococcus colony was present. Among them 3 has small and 3 has medium size There are 4 colonies of staphylococcus who has irregular shape. There are some colonies of them who are actually made their colony with the segments of bacteria streaking colony.
3. There were present 1white color of streptococcus colony with irregular shape and size was medium.
4. Around 5 colonies of fungus were present in the culture. 2 have regular shape and 3 have irregular shape. They have white color. Two of them are get touched with the streaking colony.
5. Long chain of streptococcus was present, who has flat shape.

Discussion:

“Culturing and isolation technique we have done in the aerobic environment. Through the experiment, we have seen the effect of huge number of microorganism’s colonies from the bacteria sample of hair. Aerobic bacteria culture means, the bacteria we have grow in the oxygenated environment, so that the bacteria can take energy, increase their growth, do multiplication and make the colonies. Its means the in aerobic culturing the bacteria use oxygen for their surviving and metabolism. There are bacteria that are actually aerobic like as bacillus, mycobacterium tuberculosis, lactobacillus etc”. (Sandyarani, 2019).We have done our experiment to maintain aseptic technique. As an example I can give that we have sterile our inoculating dose before and after the procedure by the Bunsen burner. Because if we did not maintain that, there will be contamination between me and other group of member samples and it will create a fault outcome in our result. So it is very important to maintain sterilization and aseptic technique.

After 24-hour observation (hair): after 24-hour experiment, we have seen that in our culture there was the growth microorganism colony. The samples bacteria (hair microorganism) I have taken it were red and put it on the A section and do streaking. And most amazing matter is that, after 24 hours as I checked, there was also a red bacteria colony. There are also a big fungus colony was present. I think that, we have cultured our sample with other sample in the same incubator machine, so that’s why it might be possible that the fungi can be migrating from other sample or it might be come from the environment. We have seen that in section C, there were fewer bacteria rather the A or B section. Because when we streaking the loop, that time the sample of bacteria get attached more with A and B section rather than section C and that’s why the number o bacteria was less in the section C and they were countable. The red chain made by the bacteria was streptococcus bacteria. There might be some staphylococcus bacteria present. I have discussed about them in the other section (environment).

After 48 observation (Hair): after 48 hours we have seen that, the line of the bacteria got totally red color. And that time the number of the colony of the bacteria is reduced. They are replicating in enough number and large in size also, that’s why they get touched with one other. The fungus colony in the section A got much bigger size than before and spread out the margins of the bacteria, because of their replication. When the microorganisms get food and proper environment they just do replication and make colonies.

After 48 hour observation (Environment): The culture we have seen through our experiment, there are some different kinds of microorganism like staphylococcus and streptococcus and fungus. The bacteria we seen except the line those might be staphylococcus. “Staphylococcus is a microorganism which one carried by the skin, Nasopharynx and gastrointestinal tract. They can be cultured from cages, room surface and personnel. They are the major group of bacteria, which can live eternal and external of a host. They are also opportunistic pathogen.” (Humphreys. 2019). Streptococcus is the types of bacteria. There are many types but among them Group A and group b can cause several types of disease like as strap throat, scarlet fever and impetigo etc. The line the bacteria maid on the agar culture, those are actually streptococcus. They are the pathogenic bacteria. They like to stay together by make the chain. As we seen in our experiment that the streptococcus makes the chain like colony from A to C section.

“By the streaking method we can get a pure sample of bacteria. The important of the streaking method is many more. By streaking method we find out the isolated organism from the blood, sputum, urine and from other things in the body and can identified the cause of the disease by isolated the sample with streaking method technique”.

Conclusion:

Streaking plate techniques is very important for isolated the microorganism colonies. Through our experiment we have learnt how we can streak on the surface of agar, the purpose of sterile the inoculating loops. “By the streaking method we can get a pure sample of bacteria. The important of the streaking method is many more. By streaking method we find out the isolated organism from the blood, sputum, urine and from other things in the body and can identified the cause of the disease by isolated the sample with streaking method technique. These techniques will help us in our future.