The Accidental Discovery

Introduction

Do you recollect from you textbooks, that microorganisms are present everywhere in nature? A variety of microbes are present in soil, air or water. Some of these produce and secrete various chemicals which might affect bacterial growth. Have you ever seen an individual or cluster of microorganisms without a microscope? In this task, we will try to grow microorganisms using a simple method. The microbes, after growth will appear like tiny dots to the naked eye. These are known as 'colonies'. A colony is formed by millions of microbial cells piled upon each other, all originating from a single cell. We will be growing microorganisms from soil sample or tap water sample onto a cooked potato slice, which would act as nutrient medium for the microorganisms.

Task 1: Become a microbiologist!

Materials required: Boiled potato, Petri dishes, test tubes, test tube stand, tissue paper, boiled and cooled water, ear buds/ cotton buds, dropper, soil sample, large tray, liquid soap/detergent.

Method

Preparation before starting the task

1. Boil a medium sized potato for 20 mins and allow it to cool completely.
2. Boil 50 ml of water for 10 mins and cool it to room temperature. This water can be referred to as sterile (microorganism-free) water. Use this water in every step where sterile water needs to be used.
3. Clean the glassware like test tubes, beakers and Petri plates with any disinfectant like detergent. Wash them properly and dry them completely.

Protocol for the task

1. Take a pinch of soil in a clean and dry test tube and add about 5 ml sterile water to it. Shake it thoroughly and leave it undisturbed for 15 mins so that the soil particles settle down.
2. Take three clean and dry Petri plates and place a tissue paper at the base of each. Use the sterile water for moistening the tissue paper. Label the plates as Plate one, plate two and plate three.
3. Take the potato and cut uniform slices such that you get discs of potato. Do not cut the slices very thin. Hold the potato slice carefully on the edges where the peel is intact and place one slice on the moist tissue paper in each Petri plate. Avoid touching the flat surface of the slice.

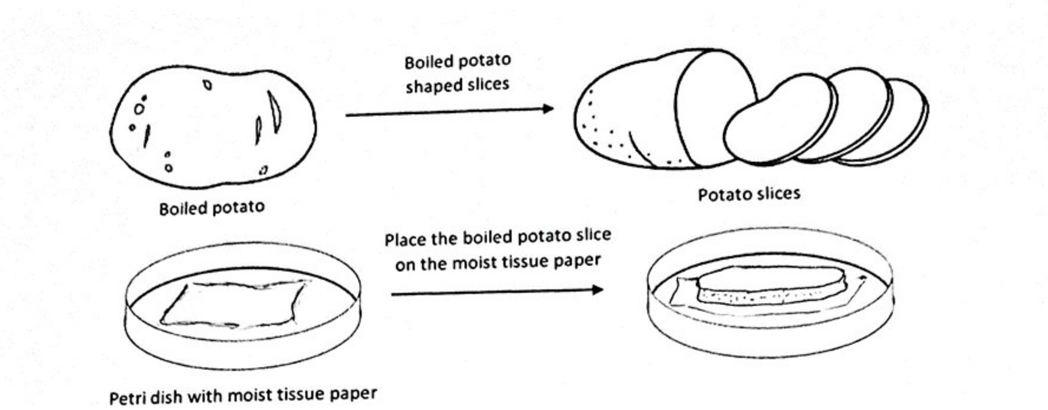

Figure 1:

4. Dip a cotton bud (you can also make a cotton bud by wrapping loose cotton around a toothpick or a small broomstick) into the soil suspension and spread it all over the surface of the potato slice in plate one. While dipping the cotton bud into the suspension, do not touch the bottom of the tube in order to avoid mixing of soil particles.
5. Take another dry cotton bud and dip it in the sterile water. Now spread it in all directions on the potato slice in plate two.
6. Do not spread anything on the potato slice in plate three.
7. Cover each potato slice with a clean and dry beaker. Keep the setup undisturbed for a day.
8. On the next day, observe the slices for any microbial growth. If the slice appears dry, add some sterile water to moisten the tissue paper beneath. Do not add the water directly on the potato slice.

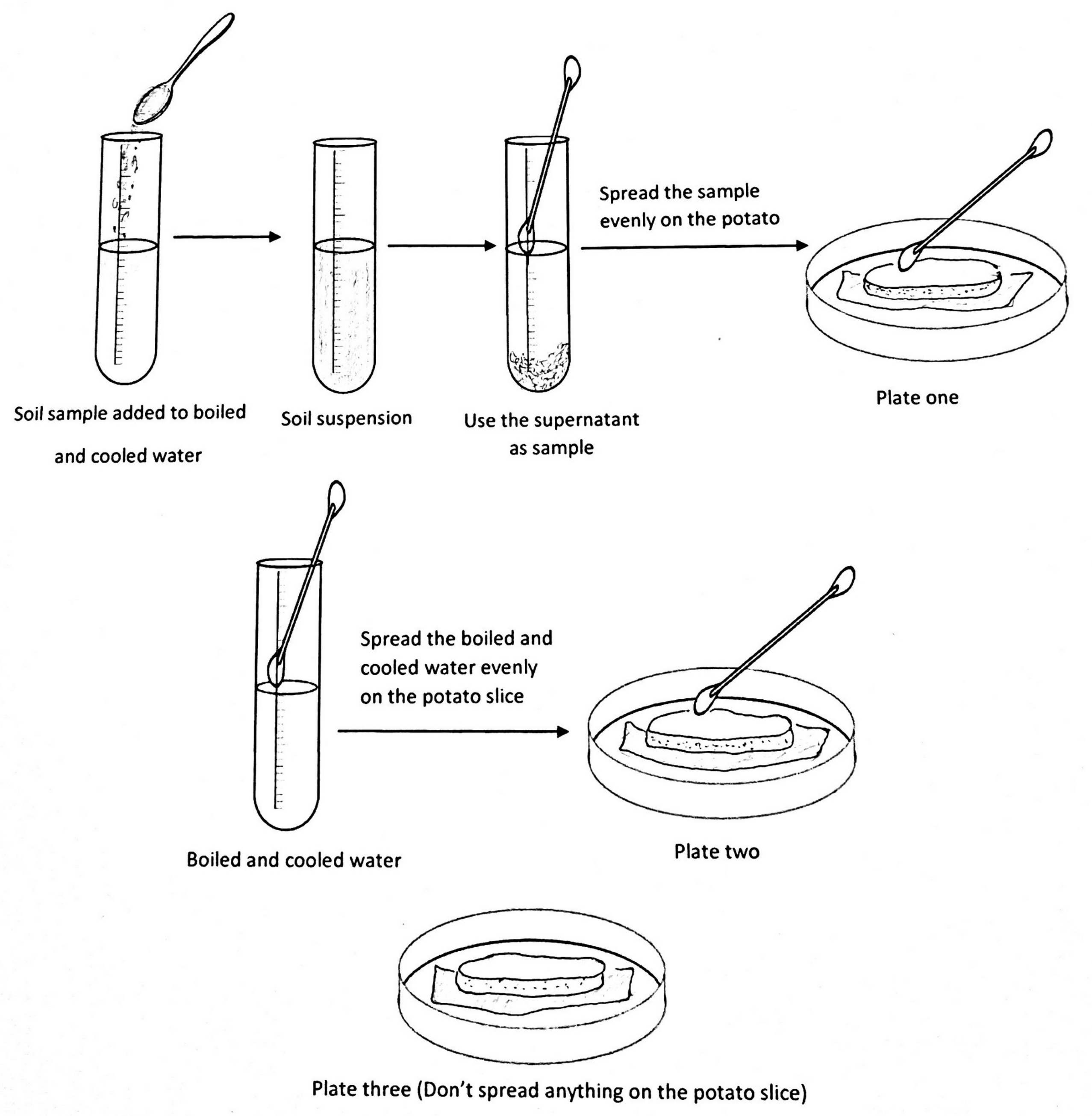

Figure 2:

9. Observe the slices carefully every day for the next two days and note down your observations in the table given below.

Observation table

Source of sample: ____

Plate	Observations (type of growth, colour, texture, etc.)	
	Day 1	Day 2
Plate one		
Plate two		
Plate three		

Q1. What do the above observations tell you?

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Q2. What is the importance of taking three potato slices and spreading the soil sample on only one of the potato slice? What is the use of the other two potato slices?

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Q3. If microorganisms are present everywhere then why don't they grow everywhere?

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Q4. After the task is over, how do you think one should dispose the potato slices, such that it does not cause any harm to anyone around them?

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Note: In this process, the potato slice is acting as a growth medium for micro-organisms, allowing the growth of multiple types of micro-organisms on it. Since these micro-organisms are not further identified and we cannot determine if they are pathogenic or non-pathogenic. Hence, in order to avoid any infection or harmful outcome proper disinfection of the potato slice is needed before disposing it. This will ensure that the micro-organisms growing on the potato slice are killed.

Do you know HOW micro-organisms are grown in laboratories?

Just like us, micro-organisms also require food. Scientists provide food, called **nutrient medium** to micro-organisms in plates called '**Petri-dishes**' like the one in the figure. These dishes have lids which can be closed and then kept in a temperature controlled closed chamber, called an **incubator**. The temperature can be adjusted to best suit the microorganisms we intend to grow.

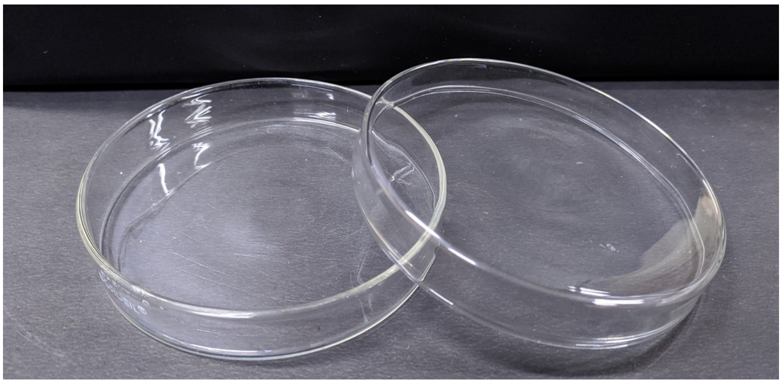


Figure 3: Petri Dish

Figure 4: Incubator

Task 2: Uncover the story!

The world is filled with problems for which humankind is struggling to find solutions. This need eventually leads to new solutions created by humankind. These newly created solutions are often referred to as inventions. But what about the solutions that already exist in our surroundings. The reality is that they usually go unnoticed. Sometimes when we look carefully and notice how useful they are as a solution to our problem, we tend to discover a new thing. These newly noticed pre-existing solutions are called discoveries.

Today we will be exploring the story behind one such discovery in the history of humankind. A discovery which would save millions of lives and give humankind a chance of survival against deadly infections.

So, let's re-discover the journey of penicillin, and how it became a life saving antibiotic.

Did you know how the first antibiotic was discovered by accident? Although the fungus (*Penicillium notatum*) that produces penicillin was discovered in 1928, its first use in humans happened only in 1941. Why did such an important discovery take so long to reach the society?

Today, a small cut or wound is not a very serious thing to happen to anyone. Some pain/discomfort can be easily managed, thanks to a range of antiseptic creams, lotions and powders available now.

Until about 90 years back, treatment of wounds was a great challenge. Even minor injuries like cuts or burns could lead to severe infections and death. Treating such patients was a challenge to hospitals, doctors and scientists all over the world.

Important: Go sequentially. If you skip the questions and read the story first, you will miss out on all the fun. So, try and answer all questions of one part before moving on to the next.
Let's begin the story behind the discovery of Penicillin

Sir Alexander Fleming was a scientist at a hospital in London. In 1928, he was growing an infectious bacterial species called *Staphylococcus* in the lab. These bacteria are commonly responsible for skin infections, sore throat or even major infections like that of the urinary tract or lungs (pneumonia).
When Alexander Fleming returned from one of his vacations in 1928, he noticed a plate of *Staphylococcus* was mistakenly left near a window. On this plate (shown below), he noticed that something other than *Staphylococcus* had also grown (image, adapted from Fleming, 1929). This growth appeared to be green in colour. This other microorganism was later identified to be the green fungus *Penicillium notatum.*

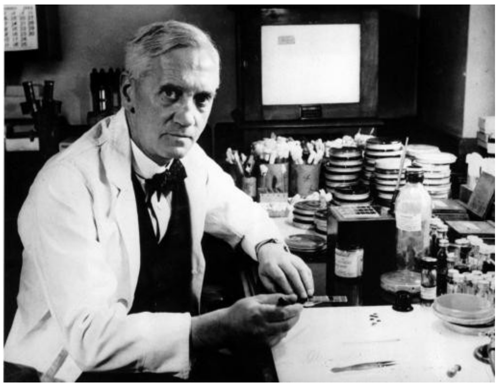
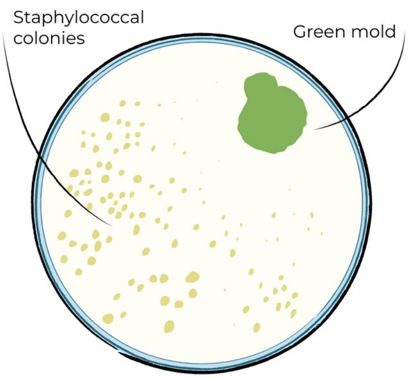
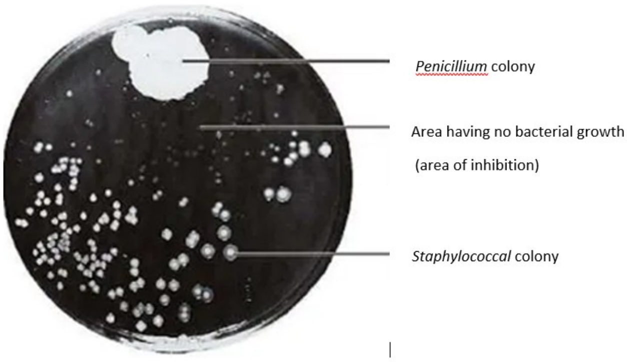


Figure 3: Alexander Fleming





Q1. What can you observe on the plate?

Q2. Have you observed any kind of a green microbial growth before? If so, where?

Q3. If Fleming was trying to grow only *Staphylococcus*, from where do you think this other microorganism entered the plate?

Q4. When Fleming looked carefully, he observed the absence of bacterial colonies closer to the fungus. That set him thinking: Why were the bacteria unable to grow in the area surrounding the fungus? Can you help him answer?

Q5. Imagine you are Fleming; how would you make use of the phenomenon: where the fungus isn't allowing bacteria to grow near it?

Fleming identified the fungus to be *Penicilium notatum.* He then grew it in a nutrient rich liquid called '**culture medium**' so that he could extract the fungus juice 'Penicillin' from it. It was this Penicillin which killed bacteria around the fungus. However, he realized that this Penicillin would quickly become inactive on storage, and hence could not be given to patients. Fleming tried several different ways to purify the active Penicillin for almost 10 years, but he was unsuccessful.

Q6.What do you think Fleming would have done then?

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Fleming gave this fungus to two other scientists: Howard Florey and Ernst Chain at Oxford University in 1939. Together with another scientist Norman Heatley, they succeeded in producing an 'active' form of Penicillin. Then, they had to check if this worked fine against infections. So, they infected some
laboratory mice with a highly infectious bacterium. Then, 50 % of these infected mice were given Penicillin while the remaining 50 % were not.

Q 7. What do you think happened to:

a) Mice which received Penicillin

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b) Mice which did not receive Penicillin

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Q 8. Why was penicillin first tried on mice and not directly used on humans?

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In 1941, a man called Albert bruised his mouth by rose thorns. The bruises slowly became a life-threatening infection. He was given Penicillin to treat it. Although he was recovering slowly, he died soon after because there was not enough Penicillin to cure him completely. This shows that a large amount of penicillin was needed for the complete treatment of infection in a single person and the current methods used were not able to produce large amounts of penicillin. Hence, it was crucial to develop new techniques for large scale production of penicillin.

The clue for excess penicillin production

Florey employed six women, who became famous as 'Penicillin Girls'.——These girls would extract hundreds of litres of mould juice just to obtain few milligrams of penicillin. Meanwhile, Heatley literally used all possible food tins, bed pans and bottles and designed 500 stackable ceramic bedpans, so that hundreds of litres could give a few grams of active Penicillin.

In spite of these procedures, the penicillin produced was not enough. Florey and Heatley were in search of ways to increase penicillin production. Did you know, that the clue for excess production of penicillin came from a spoiled fruit???

Can you find out the name of this fruit? (Hint: It is a summer fruit)

M_ _ _ _ _ L _N

War conditions had made research difficult in England. So, just before World War II, Florey and Heatley went to America so that they could produce penicillin on a large scale. One day, a laboratory assistant brought the above fruit (M_________) to the lab which was covered with a ‘golden mould’. This mould turned out to be a close relative of *Penicillium notatum* (the green mould observed by Fleming). This particular mould was called *Penicillium chrysogenum* and it yielded 200 times more penicillin than *Penicillium notatum*, the earlier mould! and helped in large scale production of penicillin.

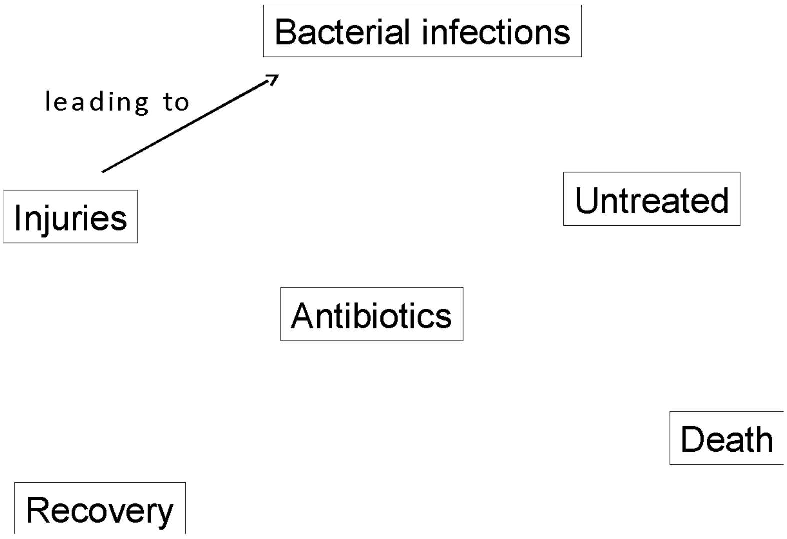
The wonder drug- Penicillin proved successful in saving the lives of many injured soldiers from bacterial infections during World War II.

Choose the Nobel laureate(s)

Imagine: You are a part of the Nobel Prize Committee. Now that you have read the entire story, who all would you award the Nobel Prize for the discovery of penicillin and why? The Nobel Prize is can be shared by not more than 3 people at a time.

The Nobel Prize was actually awarded to Alexander Fleming, Howard Florey (Pathologist) and Ernst Chain (Biochemist). The Nobel Prize cannot be shared by more than 3 people at a time.

Now, perform a task:
Make a map connecting the words below using arrows ( ). On the arrows, write the appropriate linking phrases. You may choose the linking phrases from these: **‘if left’, ‘may lead to’, ‘could result in’, ‘if treated with’** or any other phrase you find appropriate. The first one is done for you



Fun fact: Although antibiotics were not known to ancient man, Egyptians would apply a mouldy bread on infected wounds, to help them heal better. It is from such observations that we now know that certain bacteria or fungi produce compounds that can kill other micro-organisms. But now that we have finally discovered antibiotics, this method is no longer in practice. Since we are now aware that not all microorganisms are beneficial to us, it is best that we do not attempt to try this ourselves.

Think: How would a mouldy bread help in healing of wounds?

Think: How did penicillin help save millions of lives during the war? Does penicillin help in healing of wounds?

Think: How was Fleming able to produce penicillin just by growing the fungus *Penicillium notatum* in the absence of the bacteria *Staphylococcus?* Doesn't this contradict the idea that antibiotics are produced by an organism to kill the other organisms in the surrounding?