

The Accidental Discovery

Overview

This unit is based on re-living a classic experiment from the History of Science and involves two tasks: In task 1, students will try to culture and observe the microbes from soil on a potato slice. The possibility of observing the diversity of microbes around them with naked eye by culturing the microorganisms will provide a good learning opportunity for the students. This will also enable them to demonstrate the ubiquitous (present everywhere) nature of microorganisms and observe the interactions between them. In task 2, students will figure out how the ubiquitous nature of microorganisms led to one of the most popular discoveries of the millennium- antibiotics! In this task, students will be unfolding a major scientific discovery in the form of a story followed by questions for critical thinking. This will enable them to understand the importance of observation, analysis, teamwork and patience while conducting scientific experiments.

Time required:

4 sessions of 40 min each (2 sessions for each task)

Type of learning unit

Task 1: outdoors and laboratory, task 2: classroom

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.

Introduction

Microorganisms are present everywhere in nature. They are present in air, water, soil and almost on all surfaces. They are also present in extreme environments like deep seas and hot water springs. They are present on and inside the human body. Microorganisms are differently adapted to their environment for survival. They grow everywhere in nature and can also be studied in the laboratory if one is able to culture microorganisms in the lab.

In task 1, we present the students a technique to 'culture bacteria' from garden soil or from waste water sample on commonly available medium like cooked potato slices. They would be excited to see the diversity of the organisms. This will help them to further understand the ubiquitous nature of microorganisms as well as observe actual microbial growth in the form of colonies. Many types of microorganisms grow together in the same habitat. They can help each other grow together, or can be harmless to each other. Some types of organisms compete with other types in case there is a competition for nutrients. They may produce 'antibiotics' which can kill the competing microorganisms.

The first antibiotic to be discovered was penicillin from the mould *Penicillium notatum*. After the discovery of penicillin and its tremendous success in preventing bacterial infections in wounded

soldiers in World War II, newly discovered antibiotics became first line of treatment to control bacterial infections.

Scientific discoveries are often given a limited space in school textbooks. The information is restricted to the name of the scientist, a photo and few lines on the discovery. If the discoveries are read in the form of a story, as given in task 2, students would understand the aspects of nature of science, the significance of scientific processes like observation, hypothesizing, collaboration, efforts and patience that go into scientific research. More importantly, students would also realize that discoveries may happen overnight but application of the discovery may take much longer.

Learning objectives:

- To realize that observations can lead to scientific discoveries.
- To learn about substances produced by micro-organisms which can inhibit the growth of other micro-organisms. These substances are called antibiotics.
- To observe microbial colonies using simple laboratory setup without the use of a microscope.
- To understand the importance of controls to compare the results of an experiment and make a meaningful interpretation.
- To be able to culture micro-organisms using commonly available food substances as growth medium.
- To understand the need to purify and test substances for safe use in humans.
- To realize that the true potential for scientific discovery may need independent work by different groups of scientists and be spread over many decades.

Pre-requisites for the unit

Before working on the tasks, students should have some idea about pathogenic and non-pathogenic microorganisms. They should also know the difference between fungi and bacteria.

Links to the curriculum

- 1) Class 8th, Chapter 2 Microorganisms: Friend and Foe-Medicinal use of Microorganisms, panel on discovery of antibiotics.
- 2) Class 9th, Chapter 13 Why Do We Fall Ill? How antibiotics function against bacteria, Why antibiotics do not act against viruses, activity on use of antibiotics, principles of treatment.

Task 1: Become a microbiologist!

- In this task, students will try to grow bacteria on a potato slice. The source of bacteria could be fertile soil or tap water.
- For this task, students would work in groups of 3-4 persons. Encourage different groups to get soil sample from different locations.
- The task is to be done in a laboratory in presence of a teacher/facilitator.
- The experiment may require 30-40 min to perform. The slices will have to be kept in the lab for 1-2 days. Students can observe the slices for any changes after 20-24 hours.
- Teachers can click pictures of the slices after small dot-like colonies are visible on the slices.

Do you recollect from your 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.

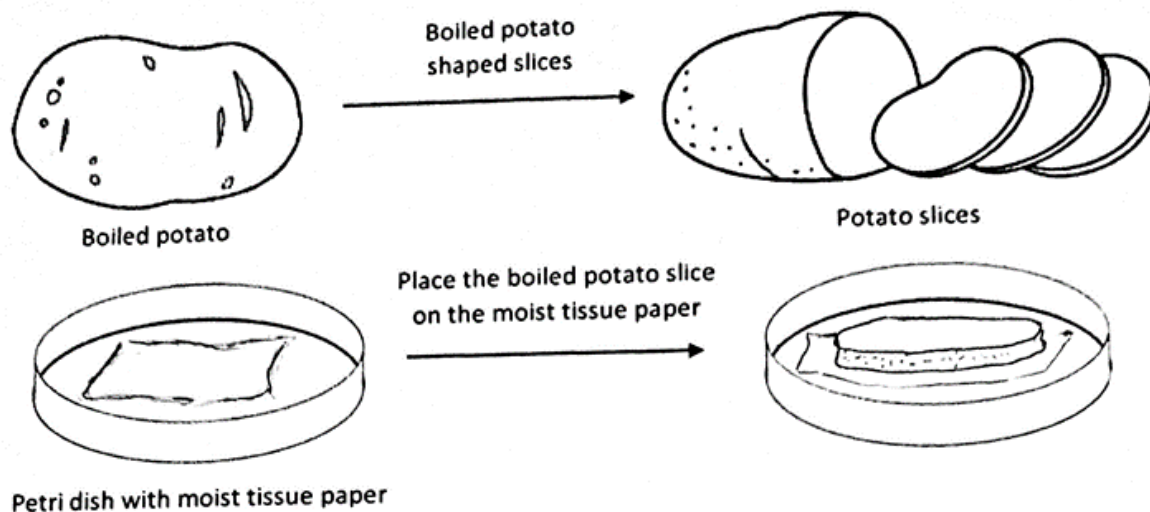
Method

Preparation before starting the task

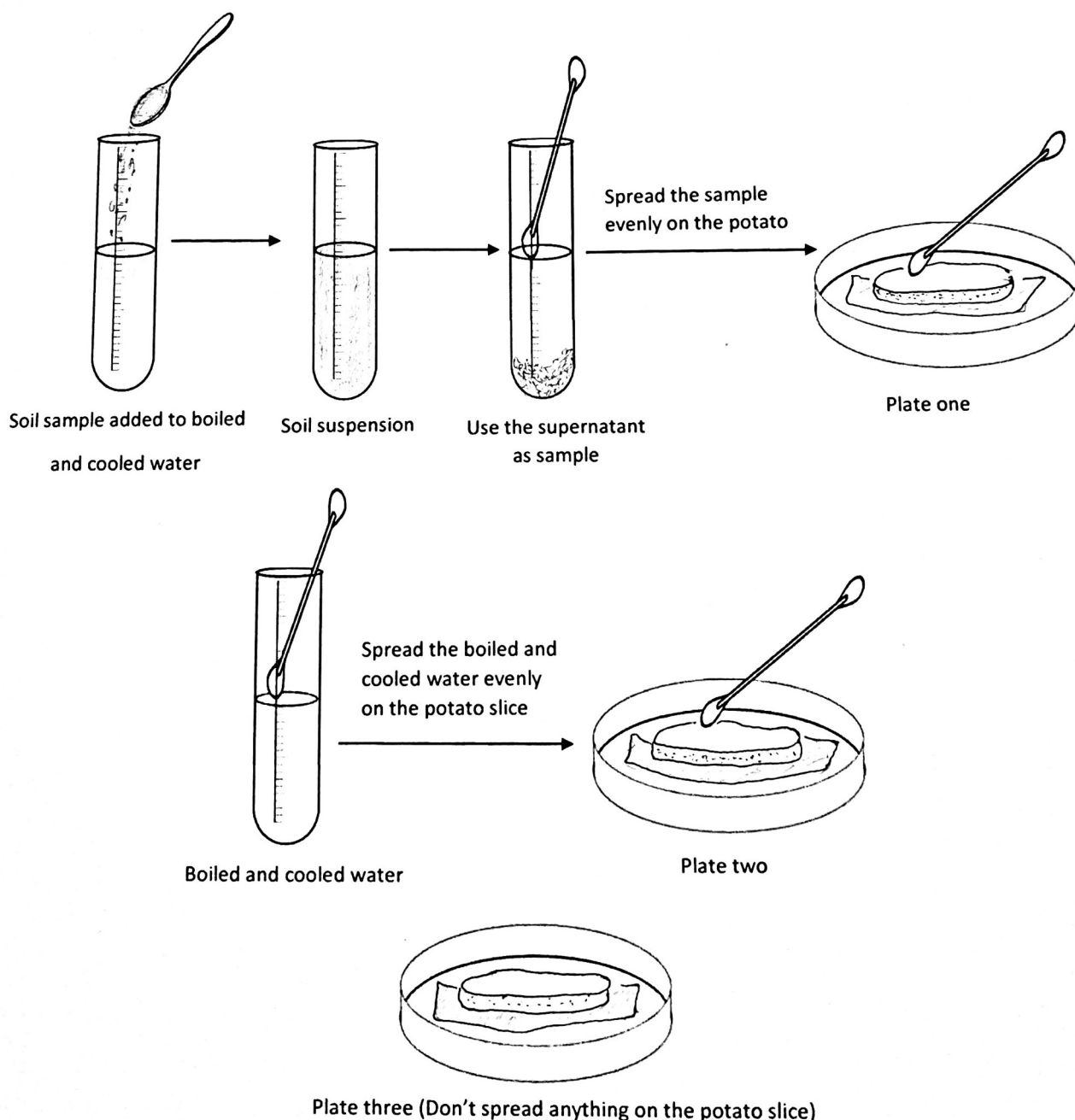
1. Boil a medium sized potato for 20 mins and allow it to cool completely.
2. Boil 50 ml of water for 10 mins and cool it to room temperature. This water can be referred to as sterile (microorganism-free) water. Use this water in every step where sterile water needs to be used.
3. Clean the glassware 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.



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.
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?

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?

The other two plates are used as controls to compare the results obtained on plate one. Plate two is used to check if the sterile water is having any microbes in it and plate three is used to check if the potato slice has any initial microbes on it. If the growth is present on plate one and absent on plate two and three, we can be sure that all the micro-organisms growing on the plate one are actually from the soil sample and not from the sterile water or potato slice.

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

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?

For disposal of the potato slices after the experiment:

- Take a large tray and add a spoonful of liquid soap/detergent powder.
- Add water to the tray and work a rich foam.
- Put all the potato slices into this tray and leave for 1-2 hours.
- Separate the water using a strainer and wrap the slices in a paper bag.
- The slices can be now disposed off as 'compostable waste'.

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.



Petri-dish



Incubator

Task 2: Uncover the story!

Begin the session with a question:

Teacher: Have you ever had an infection like a cold or cough? What medicine does the doctor give you if the infection is severe?

The students may give different answers and some of them may give the answer as antibiotics.

Teacher: What do you think is an antibiotic and who produces antibiotics?

Antibiotic is a substance or compound which was historically obtained from microbes. It kills susceptible micro-organisms or inhibits their growth. Semi-synthetic antibiotics are also available these days which are developed by chemically modifying the microbially derived antibiotic. Synthetic antibiotics are derived from non-microbial sources. Antibiotics may also be obtained from plant extracts and other natural substances.

Teacher: Would you like to know the story behind the discovery of antibiotics? Lets read then.

- Encourage maximum participation by students during the discussion.
- It is strongly recommended to not give the answer directly without any discussion.
- Consider all possible answers/responses, without classifying them as 'right/wrong'.
- The responses may be written on the board and students may themselves debate over the ideas.
- For most of the questions, there need not be one and only one right answer. Welcome all the possible responses for such questions. Other students may be asked to 'agree/disagree' with the responses along with their reasons to agree or disagree.
- It would be helpful not to be too critical or disapproving of students' views

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.

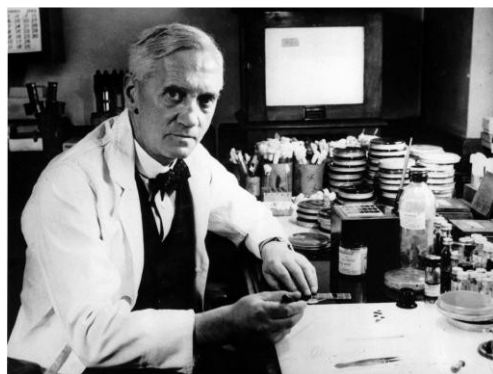
Until about 90 years back, treatment of wounds was a great challenge. Even minor injuries like cuts or burns could lead to severe infections and death. Treating such patients was a challenge to hospitals, doctors and scientists all over the world.

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*.





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?

Students know about the ubiquitous nature of micro-organisms. They know that micro-organisms are present in the air. The microorganism would have entered through air or would have already been present in the plate, but just got favourable conditions to grow as it was near the window.

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?

The fungus could have produced something which prevented the bacterial growth.

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

If the bacteria are human pathogens, i.e. causing infection in humans, the fungus can be used to kill the bacteria.

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?

Let students think of all possibilities here, like “Fleming would have asked for help, given his fungus to someone else, would have stopped working and turned his attention to something else, etc.”

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

b) Mice which did not receive Penicillin

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

Before testing any drug on humans, it is usually tested in animals such as mice, mainly to test the drug for risks such as allergies or toxicity. Mice that are specially bred for the purpose of laboratory research and are different than the ones which damage our foodstuff at home! Mice that are bred for research are easy to handle, have small gestation periods (19-20 days, as against 9 months in humans) and produce a litter of 5-8 young ones at a time. Importantly, human’s genes are very closely related to those of mice. All these factors make mice the most suitable option as model animals. If the toxicity of a drug is not known, would someone try it directly on humans?

No. But then why try it on mice either? Why kill mice? If a drug has the potential to cure a human, it could also cause death of some animals in the process of testing. But, it could become a cure and save several human lives. There are regulatory bodies such as the ‘Animal ethics committee’ which regulate the use of laboratory animals.

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)

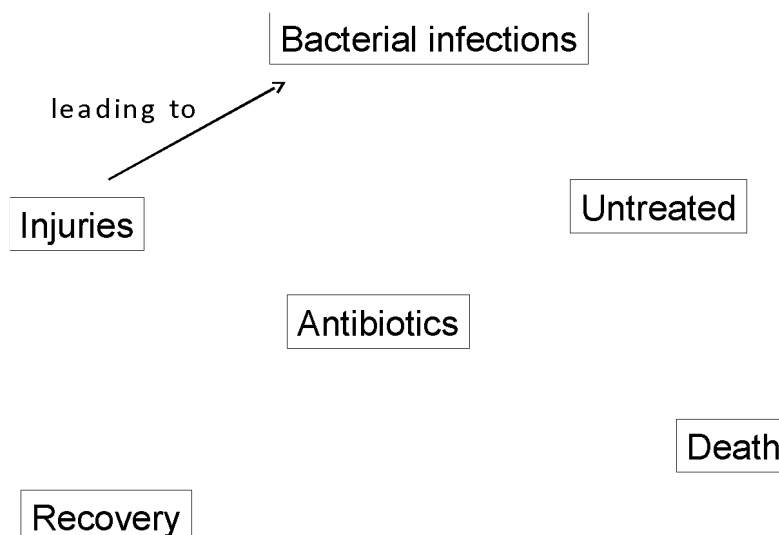
Imagine: You are a part of the Nobel Prize Committee. Now that you have read the entire story, who all would you award the Nobel Prize for the discovery of penicillin and why? The Nobel Prize is can be shared by not more than 3 people at a time.

This is an open-ended question. Allow students to discuss all possibilities.

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?

A mouldy bread consists of a mould or fungus which might be releasing some chemical to stop or kill the bacteria infecting wounds.

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

During the world-war, people could have died more of bacterial infections caused due to the wounds, then the wound itself. So, penicillin helped in preventing infections in wounded soldiers.

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?

In few species when the microbes reach a certain population and there is scarcity of nutrients in the surroundings they start to produce certain substances. Sometimes, these substances seem to inhibit the growth of other species of microbes growing in the surrounding. These substances are called antibiotics. Hence, we can say that the production of antibiotics is dependent on the population, the stage of cell cycle and the availability of nutrients rather than the presence of other species of organisms. This also answers the question as to why not all microbes produce antibiotics even though different species of microbes grow in close vicinity to each other in nature.

Once the students have solved all questions, teacher can discuss further questions (in discussion mode, writing not needed) with students. Questions for discussion are given below towards the end. We strongly recommend discussing these questions. Most of these questions are open-ended. Teachers may invite all possible answers/arguments from students and encourage students to discuss them.

1. What different take home messages did the exercise give you? (They may be asked about effect of chance, accidental discoveries, working in teams)
2. Why did we do this exercise on 'history of antibiotics'? Why is it significant now?
3. Bacteria existed even before 1928, so did fungi. So, what exactly happened in 1928, which triggered the process of purification of penicillin? (the chance event of bacteria and fungi growing on the same plate accidentally)
4. What if there was no upcoming war when penicillin was discovered?
5. If there were so many scientists involved, why is only Fleming's name so popular and not others'?

References and suggested readings

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