The Accidental Discovery

For students

Task 1: Crowded plate technique

Do you recollect from previous year's knowledge, that microorganisms are present everywhere in nature? A variety of microorganisms are present in soil, air or water. These microorganisms produce and secrete various chemicals which might affect bacterial growth.

Have you ever tried to 'grow' microorganisms in a lab? Let's try to grow them from soil or water. We will perform a simple experiment using potato, waste-water or soil, cotton buds, clean plate.

Different groups may use soil or waste-water samples collected from different places. Preferably, use fertile soil.

Method:

1. Boil a medium-size potato for 20 min and allow it to cool.
2. Take a spoonful of soil in a clean tube and add 4 ml of boiled water to it. Mix it properly with a glass rod and let it to stand for a few minutes to allow soil particles to settle down.
4. Swab a petridish or watchglass with ethanol and place a moist tissue at the base. You may use boiled and slightly warmed water to moisten the tissue paper. Make sure the water is not very hot.
5. Peel off the potato and cut 0.5 cm thick slices. Thickness of the slice should be uniform all over. Place each slice on the moist tissue paper. Each group gets 3 slices of potato.
6. Dip a cotton bud into the soil suspension / waste-water sample and spread it in all directions on the potato slice. Use different buds for different slices. Label each plate after spreading the sample with the location of soil / source of water.
7. Additionally, on another slice, spread the boiled water using an ear bud. On another slice, do not spread anything. Label these as 'controls' with and without boiled water.
8. Cover each slice with a beaker previously swabbed with ethanol or alternately cover all slices together with a large tray swabbed with ethanol. Ensure that the container is clean. Keep the setup undisturbed for a day.
9. On the next day, observe the slices for any microbial growth. If the slice appears dry, add some boiled and cooled water to moisten the tissue paper beneath.

10. Observe the slices carefully every day up to 2 days and interpret.

Q 1. What all do you see on the slice? You may draw a figure with the use of colors, if possible. Do you see a difference between day 2 and day 3?

Q 2. Why did we take a slice spread with water only and a slice on which we did not spread anything?

Q 3. How will our interpretations be affected if we don't take the slices spread with water only and another slice without spreading anything?

Record the results as per the following table:

Group no.	Sample Soil/water	Source	Observations (type of growth, colour, texture, etc.)

Take a small drop of distilled water on a slide. Add a very small part of the microbial colony to the water drop using a blunt needle. Cover the mount with a cover slip and observe under the microscope.
Take a look at other groups' mounts under the microscope.
What can you say about the diversity of colonies from various groups in your class?

Try to draw the images as you observe under the microscope.

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 lather
- Put all the potato slices into this tray and leave for 1-2 hours
- Decant the water using a strainer and wrap the slices in a paper bag
- They can be now disposed off as ‘wet waste’.

Task 2. Uncover the story!

Do you know how penicillin was discovered? The fungus *Penicillium notatum* produced the antibiotic penicillin. Although penicillin was discovered in 1928, it was administered in humans only in 1939. Why did a discovery take so long to reach the humans? We present to you a story on the discovery penicillin how it became a life-saving antibiotic. But you will uncover the story using your own thoughts and ideas!

Important: Go sequentially. If you skip the questions and read the story parts first, you will miss out on all the fun. So, try and answer all questions of one part before moving on to the next.

Have you ever seen mold growing on bread? What do you do with such a piece of bread? Ancient Egyptians would apply this moldy bread on infectious wounds to treat them. How would a mold help in healing of wounds?

Image source: Wikimedia Commons

Image source: Wikimedia Commons

Before the discovery of penicillin, minor injuries like cuts or scratches could lead to severe bacterial infections or even to death. Hospitals would be full of people with blood poisoning due to major or minor injuries.

In those days, Sir Alexander Fleming was a scientist at a hospital in London. In the year 1928, he was performing an experiment where he was growing cells of a bacteria called **Staphylococci**. For convenience, let's call it Staph!

These bacteria can cause a wide range of infections- from minor skin infections to major infections like pneumonia, toxic shock syndrome, or urinary tract infections.

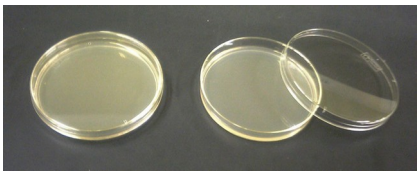
Image source: Wikimedia Commons

Do you know how bacteria are grown in laboratories?

Just like humans, bacteria also require sources of nutrients- carbon, nitrogen and salts. Bacteria, isolated from soil, water, air skin, teeth or fluids like blood, urine, stool, etc. are grown on a nutritious mixture called **culture medium** which may be liquid or solid. When the medium is solid, it is usually placed in a dish called ‘petri-plate’.

The dish has a lid and the bacteria in the plates are allowed to grow in closed plates, kept in a chamber called **incubator**.

The incubator is like a small cupboard which can maintain a set temperature. When bacteria are made to grow in a lab, the incubator is set at a specific temperature most suitable for the bacteria.



Petri-dish

Incubator

Another question, can you see a single bacterial cell with a naked eye?

What if millions of bacterial cells clump together?

Then, you can see them with a naked eye!

These millions of cells together form what is called as a ‘bacterial colony’. The size of a colony varies from a pin head to the size of a hole made by the punching machine.

Going back to 1928…

While the Staph were growing in his lab, Fleming went on a vacation and when he returned, he noticed that the plate was negligently left outside, near a window, instead of in the incubator. He observed that something other than Staph had also grown on the plate. Look at the image of the plate below.

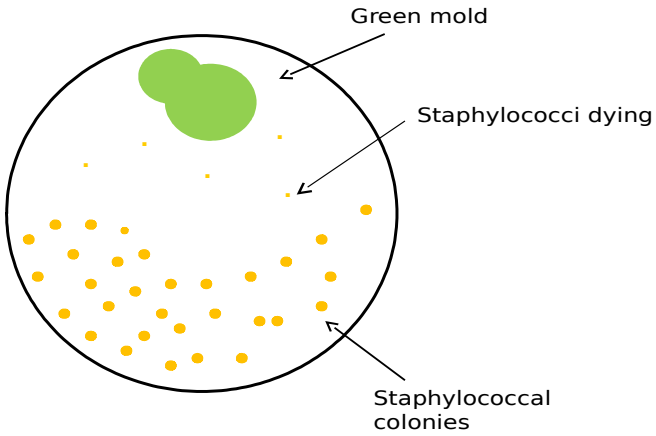


Image adapted from Fleming, 1929

Q. Have you observed this kind of a green mold before? Is mold a fungus or a bacterium? Why?

Q. If Fleming was trying to grow only bacteria, from where do you think the mold entered the plate?

Story continues….

If Fleming was trying to grow only bacteria, from where do you think the mold entered the plate? Instead of throwing the contaminated plate straight away, Fleming looked carefully and observed the absence of bacterial colonies closer to the mold. That set him thinking **why the bacteria were not able to grow near the mold**.

Q. Can you help him answer?

Q. If you were in place of Fleming, how would you make use of the process where bacteria cannot grow near a mold

Q. In the above story, suppose the plate was carefully closed and kept inside the incubator (with optimum temperature) instead of being left outside. What would have happened then?

Fleming first identified the mold to be *Penicilium notatum.* He then grew the mold in ‘culture medium’ so that he could extract the penicillin from it. It was this penicillin which killed the microbes around the mold. The extract which contained penicillin would quickly get degraded, and hence was not effective for treatment. Fleming tried different experiments to purify stable (active) penicillin for almost 10 years. But he was not successful in his attempts.

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

Story continues….

Howard Florey and Ernst Chain were scientists at Oxford university. In 1939, they borrowed Fleming’s mold. They used their knowledge of pathology and biochemistry to extract the penicillin from it. They purified penicillin by performing various experiments. They injected the extract into mice infected with another highly infectious bacterium. In their experiment, 50 % of all the mice infected with the bacterium received penicillin and the others did not.

Q. Why were only 50 % of the mice given penicillin?

Q. What do you think happened to:

a) Mice which received penicillin

_____

b) Mice which did not receive penicillin

_____

Q. Why was the experiment not tried directly in humans instead of mice?

_____

_____

_____

Story continues….

Only in 1941, for the first time, penicillin was used as an antibiotic in the treatment of a patient. This patient had a bacterial infection as he got bruised with rose thorns on his face. He was given penicillin to treat the infection. But, he died as there was not enough penicillin to cure him completely. The need at that time was to produce penicillin in large amounts.

To obtain penicillin in large amounts, another scientist- Norman Heatley in Dr. Florey's lab practically used all available containers of all sorts, even bedpans and bottles to grow the mold *Penicillium notatum* in large volumes (litres). In spite of these procedures, the penicillin produced was not enough. Florey and Heatley were in search of ways to increase penicillin production.

Image: *Penicillium notatum* in bedpans (PBS Newshour The real story behind penicillin)

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

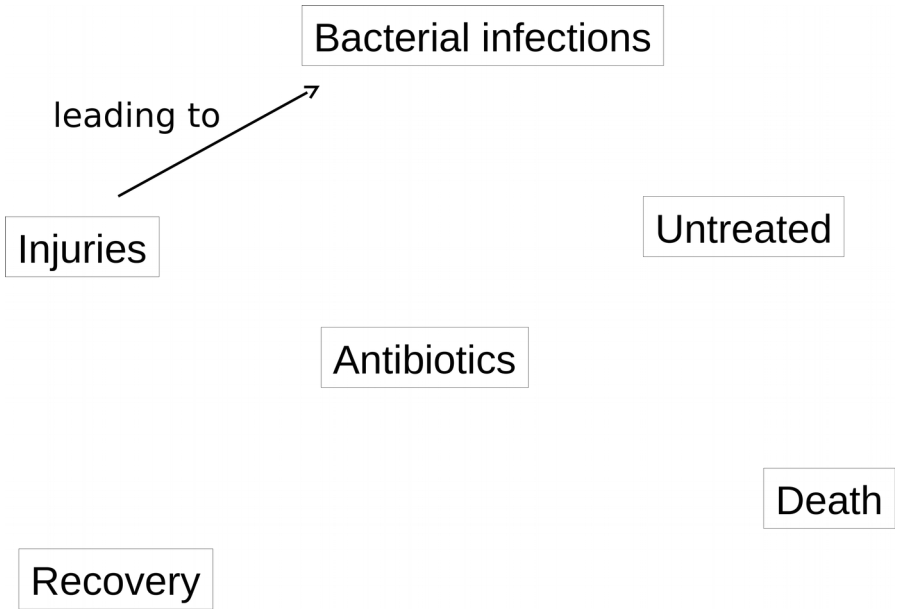
War conditions had made research difficult in England. So, just before World War II, Florey and Heatley went to America so that they could produce penicillin on a large scale. One day, a laboratory assistant brought the above fruit (M_____) to the lab which was covered with a 'golden mold'. This mold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

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

Q. When we see a fruit with a fungal growth, we usually throw it. Why did Heatley thought of using it for penicillin extraction?

Q. Imagine you are a part of the Nobel Prize Committee. Now that you have read the story, who all would you award the Nobel Prize for discovery of penicillin and why?

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



Q. In what ways did the war affect the process of this discovery?

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

Q. How will you define antibiotics?

Take-home message for you:

Complete the task using arrows as you did before to connect the words with arrows. You may use linkage words like ‘can make’, ‘lead to’, ‘need to’, ‘can be’, or any other phrase you may feel appropriate.