Miranda House
University of Delhi

INSPIRE INTERNSHIP PROGRAMME 2024

Innovation in Science Pursuits for Inspired Research
An Initiative of DST, Govt of India

8-12 JULY 2024

THE CELL:
LIFE'S PLAYGROUND

DNA:
CLOSE ENCOUNTERS

Offered by:
Botany Department

CONTENTS

WORKSHOP TITLE- THE CELL: LIFE'S PLAYGROUND

WORKSHOP TITLE- DNA: CLOSE ENCOUNTERS

A STUDY OF CERTAIN ENZYMES IN SWEET CORN GRAINS

Prof. Janaki Subramanyan, Dr. Veena Beri and Dr. Laishram Sundari Devi

Pre-activity Questions

1. What are enzymes?
2. Name some enzymes.
3. What is meant by the term substrate?
4. What is the chemical nature of enzymes?
5. How are enzymes different from other proteins?
6. Why are enzymes often referred to as biological catalysts?
7. How can we demonstrate the activity of an enzyme?
8. What is the substrate for amylase?
9. Why do we feel a sweet taste when we chew rice or a piece of bread?
10. What is the substrate for invertase?
11. What is the substrate for succinate dehydrogenase?
12. Where is succinate dehydrogenase located in the cell?
13. Give examples of enzymes having industrial applications.
14. What is sweet corn? Why is it so called?

AIM

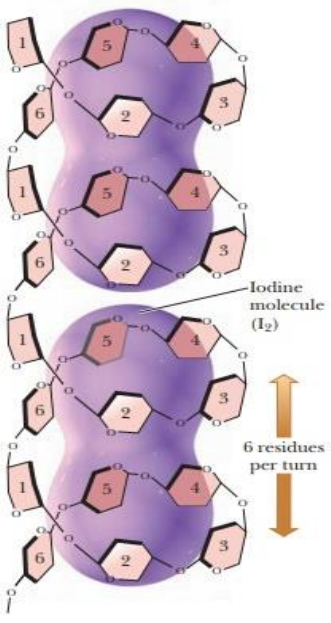
This exercise aims at detecting the activities of certain enzymes, namely amylase, invertase and succinate dehydrogenase, in sweet corn grains.

BACKGROUND

Enzymes. Enzymes, the molecular catalysts of life, are usually complex high molecular weight proteins indispensable for the several metabolic reactions that take place inside living organisms. They accelerate the rate of chemical reactions by lowering the activation energy. Enzymes act on the substrate and form the product. Every enzyme has a specific three-

dimensional structure; and enzymes are larger than their respective substrate molecules. The high specificity of enzymes can be attributed to their three-dimensional structures with the active site for binding to a particular substrate. The substrate binds to the active site of the enzyme forming a temporary complex called the enzyme-substrate complex. As the chemical reaction takes place and the products are formed, the enzyme is released unchanged from its original structure. Because the enzyme is not consumed or changed by the chemical reaction, it can be used over and over to catalyse additional substrate molecules. Enzymes function effectively under mild conditions, like physiological pH and temperature. Importantly, enzymes have several applications in industry and biotechnology.

Amylase. Amylases are hydrolytic enzymes that act on starch. Starch is a complex polysaccharide composed of amylose and amylopectin. Amylases act on starch and produce dextrins, maltose and glucose. Starch is tested using iodine reagent. Iodine reagent reacts with starch to give a blue-black complex. When starch gets degraded by amylase, the blue-black colour fades. Amylose is a left-handed helix having six glucosyl residues per turn. The coordination of iodine ions in the interior of the helix produces a blue-black colour. Starch must contain at least six helical turns, i.e., 36 glucosyl residues, to produce the blue-black colour. Amylopectin gives a red colour with iodine reagent.

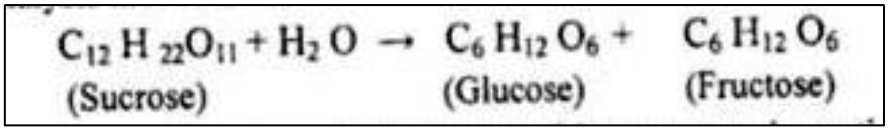


■ FIGURE 16.24 **The starch–iodine complex.** Amylose occurs as a helix with six residues per turn. In the starch–iodine complex, the iodine molecules are parallel to the long axis of the helix. Four turns of the helix are shown here. Six turns of the helix, containing 36 glycosyl residues, are required to produce the characteristic blue color of the complex.

The degradation of starch by amylase present in corn grains can be demonstrated by observing the gradual fading of the starch-iodine complex.

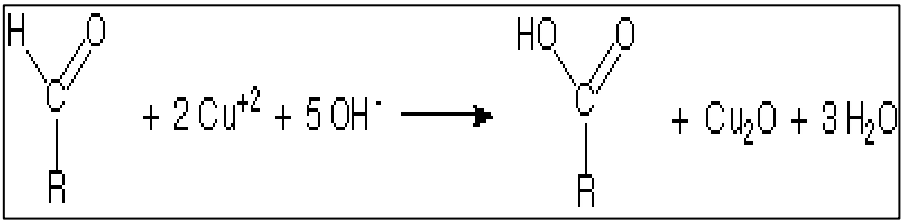
The starch-iodine complex (Photo credit: https://www.brainkart.com/article/Structures-and-Functions-of-Polysaccharides_27634/).

Invertase. Invertase is a hydrolase, cleaving the disaccharide sucrose into two monosaccharides, namely glucose and fructose. In plant cells, invertase is present in the apoplast (cell wall), vacuole and cytosol. Sucrose is a non-reducing sugar, whereas glucose and fructose are reducing sugars.



The specific rotation of sucrose is + 66.5°. The specific rotation of glucose is + 52.7 ° and of fructose is - 92 °; the specific rotation of the mixture of glucose and fructose formed is – 39 °.

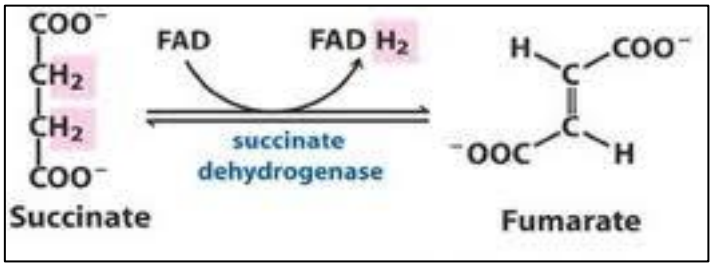
Benedict's reagent (alkaline $CuSO_4$) is used to detect reducing sugars. Reducing sugars react with Benedict's reagent to form cuprous oxide which is a brick red precipitate.



Invertase activity can be detected by conducting Benedict's test using the enzyme-substrate mixture following incubation of the enzyme extract and sucrose for a known period of time, say 15 minutes.

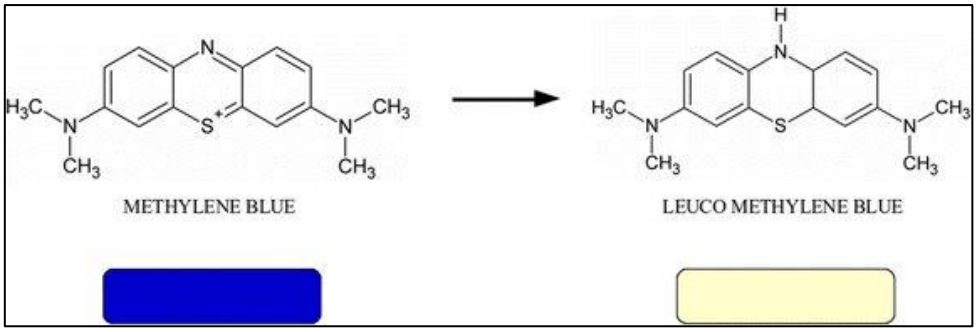
Succinate Dehydrogenase. Dehydrogenases are oxidoreductases. More than 200 dehydrogenases are known. By moving a hydride ion (H^-) from a substrate to a coenzyme, usually flavin adenine dinucleotide (FAD) or nicotinamide adenine dinucleotide (NAD^+), dehydrogenases catalyze oxidation-reduction reactions and produce $FADH_2$ or NADH, respectively. These enzymes are essential for maintaining the balance of redox reactions in cells and for regulating metabolic pathways to meet the energy demands of the organism.

Succinate dehydrogenase is tightly bound to the inner membrane of mitochondria in eukaryotes. In prokaryotes, the enzyme is bound to the plasma membrane. In cellular respiration, succinate dehydrogenase is essential for the citric acid cycle (Krebs cycle) as well as electron transport chain (ETC). The enzyme catalyses the oxidation of succinate to fumarate in the citric acid cycle. This reaction is coupled with the reduction of FAD to $FADH_2$; the electrons ultimately reach the electron carrier ubiquinone in the ETC reducing it to ubiquinol.



The oxidation of succinate to fumarate will be studied using the artificial electron acceptor methylene blue. Methylene blue is a dye that is blue in the oxidized state and colourless in the reduced state. When succinate is oxidized as a result of dehydrogenase activity, hydride ions

are released. These hydride ions would be accepted by methylene blue causing the fading of the blue colour of the dye.



The dye methylene blue in the oxidized (blue) and reduced (colourless) states (Photo credit: https://www.researchgate.net/publication/281910328_DREAM_Assay_for_Studying_Microbial_Electron_Transfer/figures?lo=1).

Succinate dehydrogenase activity can be demonstrated by observing the gradual fading of the blue colour of the dye methylene blue in the reaction mixture.

REQUIREMENTS

Plant Material: Fresh grains of sweet corn (*Zea mays*)

Glassware: Test tubes, pipettes, burettes, conical flasks, beakers, dropper, Petri dish, microslide

Chemicals: 0.5 % starch, iodine reagent, 0.5 % sucrose, Benedict's reagent, 0.05 M succinic acid, 1 % methylene blue, distilled water

Miscellaneous: Clamp stand, test tube stands, marker pen, aluminium foil, dispensers, burner, mixer-grinder, plastic strainer/ muslin cloth, blade

PROCEDURE

Fresh sweet corn grains (20 g) were ground in distilled water (100 mL) in a mixer-grinder. The homogenate was filtered through a plastic strainer or muslin cloth and diluted suitably (1 part of filtrate: 3 parts distilled water; v:v) and used as the crude enzyme extract for amylase and invertase. For detecting succinate dehydrogenase activity fresh sweet corn grains were placed on a microslide and cut longitudinally into two halves using a clean blade and used.

The enzyme extract, substrates and distilled water were taken is separate burettes. Alternatively, the enzyme extract, substrates and distilled water can be dispensed using pipettes with dispensers. The test tubes were labelled. Test tube 1 contained the enzyme and the substrate. The controls, without the substrate or the enzyme, were maintained as test tubes 2 and 3. The experiments were set up as given in the tables.

OBSERVATIONS

Demonstration of Amylase Activity

Tube No.	Enzyme extract (mL)	Starch (0.5 %, mL)	Distilled water (mL)	Iodine reagent (drops)	Intensity of blue-black colour after 10 min (+)
1	2	2	--	2	+ 2
2	2	--	2	2	Blue-black colour faded very quickly on adding iodine reagent
3	--	2	2	2	+15

Demonstration of Invertase Activity

Tube No.	Enzyme extract (mL)	Sucrose (0.5 %, mL)	Distilled water (mL)	Benedict's reagent (mL)*	Amount of ppt. formed (+)
1	2	2	--	2	+5
2	2	--	2	2	+3
3	--	2	2	2	--

** Benedict's reagent was added following incubation of the enzyme- substrate mixture for 15 minutes, and the test tubes were warmed.*

Demonstration of Succinate Dehydrogenase Activity*

Tube No.	Succinic acid (0.05 M, mL)	Distilled water (mL)	Methylene blue (1%, drops)	Five sweet corn grains, each cut longitudinally into two halves	Intensity of blue colour (+)	
					At zero min	After 40 min
1	5	--	1	Added	+6	+3
2	--	5	1	Added	+6	+3
3	5	--	1	Not added	+6	+6

** Succinate dehydrogenase activity takes time and the visual difference in the blue colour intensity between test tubes 1 and 2 will be evident only after around 2 hours from incubation.*

EXPECTED RESULTS

Amylase. Sweet corn grains have amylase activity because the first tube shows fading of the blue-black colour of starch-iodine complex with the progress of time. In this tube amylase is acting on the external as well as the internal substrate. In the second tube amylase is acting

only on the internal starch. Very little stored starch is present in the grains because most of the starch has already been converted to sugar. In the third tube the blue-black colour persists because there is no enzyme to degrade the starch.

Invertase. The first and second tubes show the formation of a brick red precipitate, which shows that the reaction mixtures contain reducing sugars. In the first tube reducing sugars already present in the enzyme extract as well as the reducing sugars formed as a result of invertase in the enzyme extract acting on the added sucrose will react with Benedict's reagent and form the brick red precipitate. However, in the second tube only the reducing sugars present in the enzyme extract will react with Benedict's reagent and form the brick red precipitate. No brick red precipitate will be formed in the third tube because the reaction mixture did not contain the enzyme extract to act on sucrose.

Succinate Dehydrogenase. The blue colour of methylene blue would fade in the first and second test tubes. However, with a prolonged incubation, say two hours or more, the first tube will show less intense blue colour than the second tube because succinic acid will be acted upon by succinate dehydrogenase. In addition to succinate dehydrogenase, the activity of other dehydrogenases in the tissue will also be taking place in tube one. The second tube would show a relatively slow fading because of the absence of succinic acid, the artificial electron donor, in the mixture. Nevertheless, the fading of methylene blue that is observed in the second tube is because of the dehydrogenases that are commonly present in all tissues. In the third tube the blue colour would remain as intense as at zero minute because sweet corn grains, the source of dehydrogenases, have not been added to the reaction mixture.

PRECAUTIONS

1. Fresh sweet corn grains should be used.
2. The enzyme extract should be freshly prepared and suitably diluted.
3. All substrates should be freshly prepared.
4. Pipetting/ dispensing using a burette should be accurate.
5. A dispenser should be fixed to the pipettes while pipetting out the solutions.
6. The test tubes should be labelled.
7. The mouths of the test tubes should be covered with aluminium foil.
8. The test tubes should be shaken after adding all the ingredients.
9. A test tube holder should be used while warming the test tubes.
10. While warming a test tube over the flame of a burner, the mouth of the test tube should not face anybody.
11. Glassware should be clean and dry.

Post-activity Questions

1. What is the grain of sweet corn?

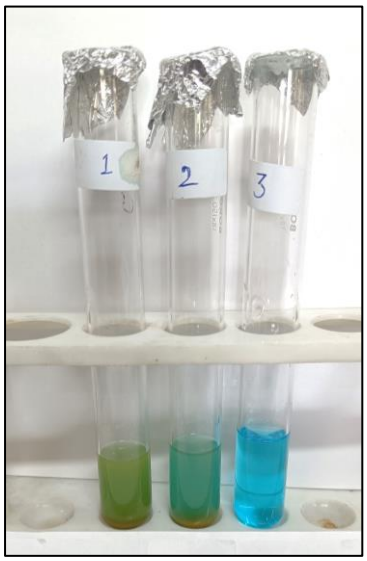
2. Name the digestive enzymes present in sweet corn grains.

3. What parts of our body produce amylase?
4. Explain the basis of the iodine test to detect starch.
5. How is a solution of 0.5 % starch prepared?
6. Why is invertase so called?
7. Will the boiled enzyme extract or boiled sweet corn grains show any enzyme activity? If no, explain why.
8. How are amylase and invertase similar?
9. Where is succinate dehydrogenase located in a cell?
10. Explain the unique dual function of succinate dehydrogenase.
11. Explain why methylene blue will not fade if corn grains are not present in the reaction mixture.
12. How can the experiment on amylase and succinate dehydrogenase be improved?

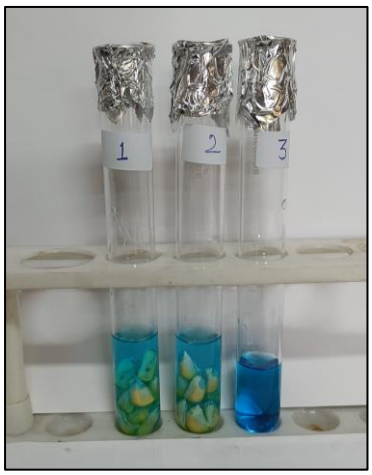
Detection of Certain Enzymes in Sweet Corn Grains

Test Tubes 2 and 3 are the controls for the enzymes being studied. Test Tube 2 is without the substrate, and Test Tube 3 is without the enzyme.

Detection of amylase activity using iodine test for starch: The fading of blue-black colour is evident in Test Tube 1. In Test Tube 2 the blue-black colour faded very quickly which showed that the enzyme extract is not rich in starch: most of the stored starch has already been converted to sugar. The reaction mixture in Test Tube 3 remained intense blue-black because there was no enzyme to act on starch.



Detection of invertase activity using Benedict's test for reducing sugars: Note the brick red precipitate formed in Test Tubes 1 and 2. The precipitate is more in Test Tube1 than in Test Tube 2. Some amount of the brick red precipitate in Test Tube 1 is still suspended and has not settled down in the reaction mixture.



Detection of succinate dehydrogenase activity: Test Tubes 1 and 2 show the fading of methylene blue because of dehydrogenases present in the cut corn grains. The presence of succinic acid in Test Tube 1 will promote succinate dehydrogenase activity; the blue colour intensity will be less in Test Tube 1 than in Test Tube 2 after a prolonged period of incubation.

MITOSIS: LOOKING INTO HOW LIVING BEINGS GROW

Dr. Saloni Bahri and Dr. Somdutta Sinha Roy

Pre-activity Questions

1. What is a cell?
2. What are the differences between plant and animal cells?
3. What is DNA?
4. What is chromatin material? Chromosomes and chromatid?
5. What is cell division?
6. Fundamental genetic material of the cell is DNA, where is it located?
7. What is the difference between a prokaryotic cell and a eukaryotic cell?
8. Which sub cellular structure is composed of proteins like microfilaments and microtubules?
9. In addition to nucleus, which other cell organelles are known to have DNA?
10. How does living organisms have genetically identical cells in their whole body?

Understanding Mitosis:

Mitosis is the process in which a eukaryotic cell nucleus splits in two, followed by division of the parent cell into two daughter cells. The word "mitosis" means "threads," and it refers to the threadlike appearance of chromosomes as the cell prepares to divide[3].

The **cell cycle** is an ordered series of events involving cell growth and cell division that produces two new daughter cells. Cells on the path to cell division proceed through a series of precisely timed and carefully regulated stages of growth, DNA replication, and division that produce two genetically identical cells. The cell cycle has two major phases: interphase and the mitotic phase or mitosis. During **interphase**, the cell grows and DNA is replicated. During the **mitosis**, the replicated DNA and cytoplasmic contents are separated and the cell divides[1].

Interphase

During interphase, the cell undergoes normal processes while also preparing for cell division. For a cell to move from interphase to the mitotic phase, many internal and external conditions must be met. The three stages of interphase are called G_1, S, and G_2 [1].

G_1 Phase

The first stage of interphase is called the **G_1 phase**, or first gap, because little change is visible. However, during the G_1 stage, the cell is quite active at the biochemical level. The cell is accumulating the building blocks of chromosomal DNA and the associated proteins, as well as accumulating enough energy reserves to complete the task of replicating each chromosome in the nucleus [1].

S Phase

Throughout interphase, nuclear DNA remains in a semi-condensed chromatin configuration. In the **S phase** (synthesis phase), DNA replication results in the formation of two identical copies of each chromosome—sister chromatids—that are firmly attached at the centromere region. At this stage, each chromosome is made of two sister chromatids and is a duplicated chromosome. The centrosome is duplicated during the S phase. The two centrosomes will give rise to the **mitotic spindle**, the apparatus that orchestrates the movement of chromosomes during mitosis. The centrosome consists of a pair of rod-like **centrioles** at right angles to each other. Centrioles help organize cell division. Centrioles are not present in the centrosomes of many eukaryotic species, such as plants and most fungi [1].

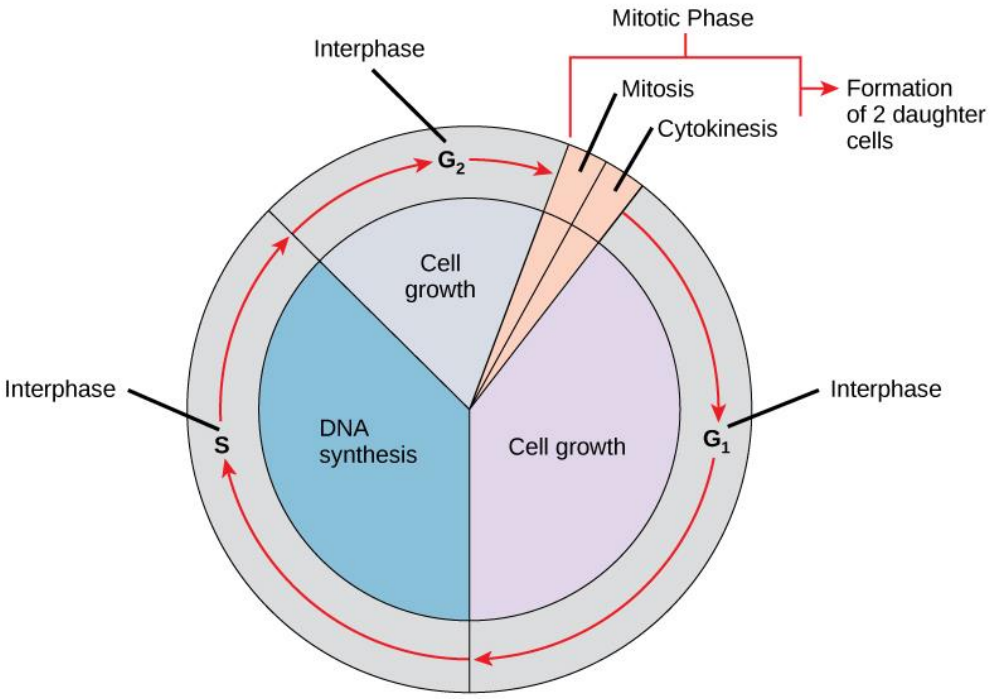


Figure 1. The Stages of Interphase and the Cell Cycle

Image credit: "The cell cycle: Figure 1"(Opens in a new window) by OpenStax College, Biology (CC BY 3.0).[2]

G_2 Phase

In the **G_2 phase**, or second gap, the cell replenishes its energy stores and synthesizes the proteins necessary for chromosome manipulation. Some cell organelles are duplicated, and the cytoskeleton is dismantled to provide resources for the mitotic spindle. There may be additional cell growth during G_2. The final preparations for the mitotic phase must be completed before the cell is able to enter the first stage of mitosis [1].

The Mitotic Phase

To make two daughter cells, the contents of the nucleus and the cytoplasm must be divided. The mitotic phase is a multistep process during which the duplicated chromosomes are aligned, separated, and moved to opposite poles of the cell, and then the cell is divided into two new identical daughter cells. The first portion of the mitotic phase, **mitosis**, is composed of five

stages, which accomplish nuclear division. The second portion of the mitotic phase, called cytokinesis, is the physical separation of the cytoplasmic components into two daughter cells. Mitosis is divided into a series of phases-prophase, prometaphase, metaphase, anaphase, and telophase-that result in the division of the cell nucleus [1].

What Happens during Prophase?

Prophase is the first stage in mitosis, occurring after the conclusion of the G_2 portion of interphase. During prophase, the parent cell chromosomes — which were duplicated during S phase — condense and become thousands of times more compact than they were during interphase. Because each duplicated chromosome consists of two identical **sister chromatids** joined at a point called the **centromere**, these structures now appear as X-shaped bodies when viewed under a microscope. Several DNA binding proteins catalyze the condensation process, including **cohesin** and **condensin**. Cohesin forms rings that hold the sister chromatids together, whereas condensin forms rings that coil the chromosomes into highly compact forms. The mitotic spindle also begins to develop during prophase. As the cell's two centrosomes move toward opposite poles, microtubules gradually assemble between them, forming the network that will later pull the duplicated chromosomes apart[3].

What Happens during Prometaphase?

When prophase is complete, the cell enters **prometaphase** - the second stage of mitosis. During prometaphase, phosphorylation of nuclear lamins by M-CDK causes the nuclear membrane to break down into numerous small vesicles. As a result, the spindle microtubules now have direct access to the genetic material of the cell. Each microtubule is highly dynamic, growing outward from the centrosome and collapsing backward as it tries to locate a chromosome. Eventually, the microtubules find their targets and connect to each chromosome at its **kinetochore**, a complex of proteins positioned at the centromere. The actual number of microtubules that attach to a kinetochore varies between species, but at least one microtubule from each pole attaches to the kinetochore of each chromosome. A tug-of-war then ensues as the chromosomes move back and forth toward the two poles [3].

What Happens during Metaphase?

As prometaphase ends and **metaphase** begins, the chromosomes align along the cell equator. Every chromosome has at least two microtubules extending from its kinetochore — with at least one microtubule connected to each pole. At this point, the tension within the cell becomes balanced, and the chromosomes no longer move back and forth. In addition, the spindle is now complete, and three groups of spindle microtubules are apparent. **Kinetochore microtubules** attach the chromosomes to the spindle pole; **interpolar microtubules** extend from the spindle pole across the equator, almost to the opposite spindle pole; and **astral microtubules** extend from the spindle pole to the cell membrane [3].

What Happens during Anaphase?

Metaphase leads to **anaphase**, during which each chromosome's sister chromatids separate and move to opposite poles of the cell. Enzymatic breakdown of cohesin - which linked the sister chromatids together during prophase -causes this separation to occur. Upon separation, every chromatid becomes an independent chromosome. Meanwhile, changes in microtubule length provide the mechanism for chromosome movement. More specifically, in the first part of anaphase -sometimes called **anaphase A**-the kinetochore microtubules shorten and draw the chromosomes toward the spindle poles. Then, in the second part of anaphase-sometimes called **anaphase B**- the astral microtubules that are anchored to the cell membrane pull the

poles further apart and the interpolar microtubules slide past each other, exerting additional pull on the chromosomes [3].

What Happens during Telophase?

During **telophase**, the chromosomes arrive at the cell poles, the mitotic spindle disassembles, and the vesicles that contain fragments of the original nuclear membrane assemble around the two sets of chromosomes. Phosphatases then dephosphorylate the lamins at each end of the cell. This dephosphorylation results in the formation of a new nuclear membrane around each group of chromosomes [3].

When Do Cells Actually Divide?

Cytokinesis is the physical process that finally splits the parent cell into two identical daughter cells. During cytokinesis, the cell membrane pinches in at the cell equator, forming a cleft called the **cleavage furrow**. The position of the furrow depends on the position of the astral and interpolar microtubules during anaphase.

The cleavage furrow forms because of the action of a contractile ring of overlapping actin and myosin filaments. As the actin and myosin filaments move past each other, the contractile ring becomes smaller, akin to pulling a drawstring at the top of a purse. When the ring reaches its smallest point, the cleavage furrow completely bisects the cell at its center, resulting in two separate daughter cells of equal size [3].

LAB EXERCISE

AIM: To study mitosis using growing root tips of Onion (*Allium cepa*).

REQUIREMENTS:

Glassware and Apparatus: Microslides, coverslip, compound microscope

Chemicals: Cornia fluid (45% Acetic acid + Ammonium chloride), acetocarmine

Plant material: Growing root tips of onion (*Allium cepa*)

Miscellaneous: Needles, forceps, filter paper, dropper, burner, match box

PROCEDURE:

1. Take the root tips of onion on a clean slide.
2. Put few drops of cornia fluid on the root tips. Tease out the root tips with the help of needles and wait for 2-3 minutes.
3. Drain out the cornia fluid. Put a drop of acetocarmine and cover it with a coverslip.
4. Very gently tap over the coverslip with the back of the needle or a pencil for better separation.
5. Observe very carefully under the microscope.

OBSERVATIONS:

Try to locate the cells with prominent chromosomes and identify the stage of mitosis from the figure given.

PRECAUTIONS:

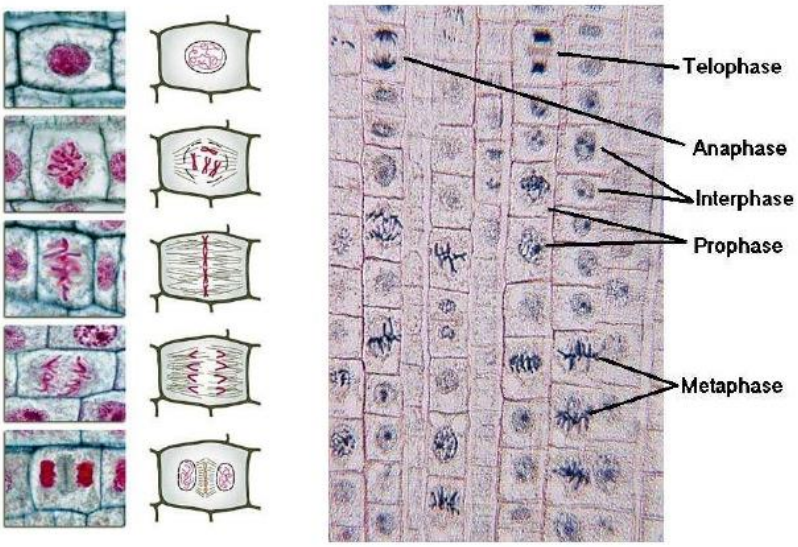
1. No water should be added.
2. Material should be teased properly.
3. Material should be left in the cornia fluid for about 5 min.
4. Tapping over the cove slip should be done gently.
5. Staining should be done carefully. Both overstaining and understaining should be avoided.
6. If heating the slide with the stain, it should be done for short time.
7. Slide should not be allowed to become dry before taking observations.
8. No stain should be flowing out of the coverslip, this can spoil the lenses of the microscope.
9. Slide should be first observed under low power of the microscope before moving to the high power.

Post-activity Questions

1. In which stage of mitosis does the nuclear membrane begin to disappear and chromatin coils into double chromosomes?
2. What is a centromere? A chromatid? Spindle fibers / microtubules? Centrioles / Asters?
3. In which stage of mitosis do the double chromosomes line up single file down the equatorial line of the cell?
4. What moves the double chromosomes around and where does it attach to them?
5. In which stage of mitosis do spindle fibers contract and pull double chromosome chromatids apart into individual chromosomes and pull them to opposite sides of the cell?
6. In which stage of mitosis does the nuclear membrane begin to reappear and chromatids/single chromosomes uncoil into chromatin strands again?
7. What is cytokinesis?
8. How do the steps in binary fission differ from MITOSIS even though both are ASEXUAL reproduction?
9. Why is fission a faster process?
10. Mitosis begins with a diploid cell and ends with how many cells of what ploidy?

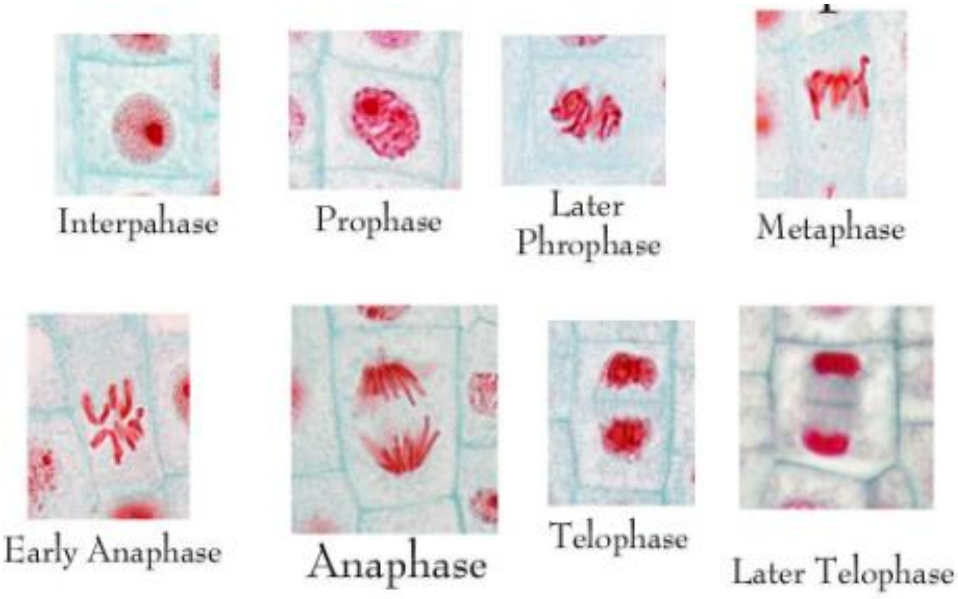
References:

[1] https://opentextbc.ca/biology/chapter/6-2-the-cell-cycle/
[2] "The cell cycle: Figure 1"(Opens in a new window) by OpenStax College, Biology (CC BY 3.0).
[3] https://www.nature.com/scitable/topicpage/mitosis-14046258/
[4] https://www.pinterest.com/pin/mitosis-in-onion-root-tips-19-cell-division-mitosis-20--279082508144714691/
[5] https://search.library.wisc.edu/digital/AVDHNYM4UFGSE487



(a)

Source: https://www.pinterest.com/pin/mitosis-in-onion-root-tips-19-cell-division-mitosis-20--279082508144714691/ [4]



https://search.library.wisc.edu/digital/AVDHNYM4UFGSE487 [5]

(b)

Figure 2 (a) and (b) : Mitosis in onion root tip cells

AZOLLA - ANABAENA: A PERFECT SYMBIOTIC PARTNERSHIP

Dr. Deepali, Dr. Elangbam Geetanjali and Dr. Rashmi Shakya

Pre-activity Questions

1. What are the sustainable agricultural practices?
2. What is the latest hot burning topic of group discussion among common man?
3. India being an agricultural country- how can we boost its agricultural productivity?
4. What are fertilizers?
5. What are the merits and demerits associated with the use of fertilizers?
6. Have you heard of the term Green Revolution? What is Green Revolution?
7. Do organisms interact with one another? If yes how?
8. What is symbiosis?
9. Have you ever heard of blue green algae? If yes, in what context?
10. Can interaction between the organisms be used as a means to protect the environment?

Biofertilizers:

A biofertilizer is a substance which contains living micro-organisms which, when applied to seeds, plant surfaces, or soil, colonize the rhizosphere or the interior of the plant and promotes growth by increasing the supply or availability of primary nutrients to the host plant and useful for sustainable agriculture.

***Azolla-Anabaena* : A perfect symbiotic partner**

Azolla, an aquatic fern, is a genus comprised of seven species that belongs to the family Salviniaceae. These are extremely reduced and specialized life forms and shows resemblance to the duckweed or water lens, the free-floating aquatic plant that floats on the surface. They do not resemble typical ferns. The common name of *Azolla* is mosquito fern, fairy moss and water fern.

. **Figure 1**: Photograph of *Azolla* growing in water (L) and a details of *Azolla* plant (R)

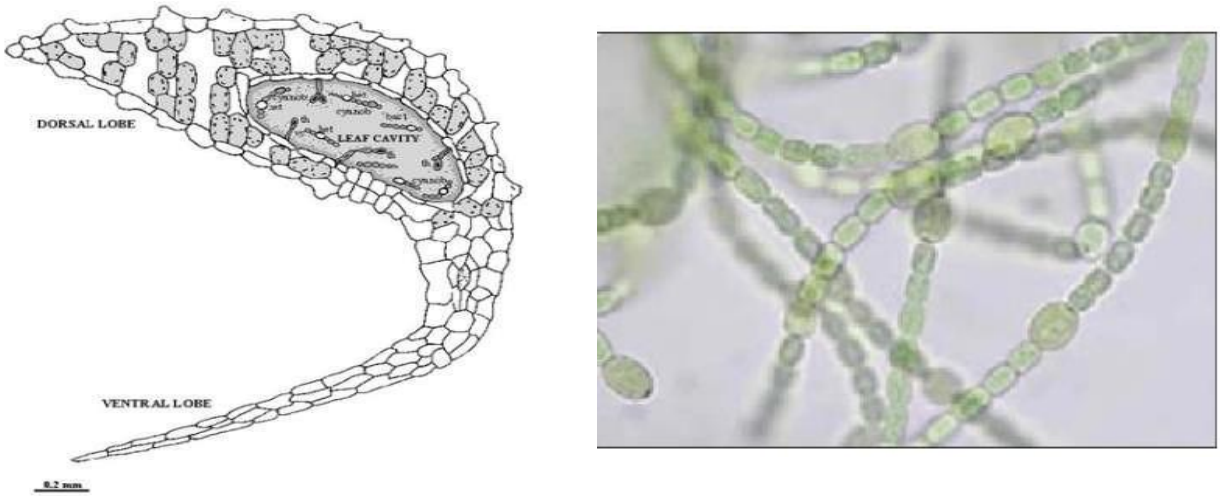


Figure 2: A diagrammatic presentation of *Azolla* frond (L) and WM of *Anabaena* filaments

A total of seven species of *Azolla* are known so far *viz*., *A. caroliniana*, *A. filiculoides, A. microphylla, A mexicana. nilotica, A. pinnata* and *A. rubra.* The common Indian species is *A. pinnata* and its fronds consist of sporophyte with a floating rhizome and small overlapping bi-lobed leaves and roots. *Anabaena*, a blue-green alga that fixes nitrogen gas, is a cyanobacterium with which *Azolla* shares a special and mutually advantageous "symbiotic relationship." For its symbiotic companion *Anabaena*, it has a special leaf structure. Despite the fact that they are both photoautotrophs, they coexist in a special "relationship" that benefits both parties. *Anabaena* gives *Azolla* a confined space in exchange for Anabaena directly sequestering atmospheric nitrogen, which *Azolla* needs for growth. For 70 million years, *Azolla* and *Anabaena* have never been separated. Over this enormous amount of time, the two partners have jointly evolved a variety of complimentary behaviors that increase their resourcefulness. *Azolla* is able to generate enormous amounts of biofertilizers because of this. *Azolla* leaves are made up of a thinner, translucent ventral (lower) lobe submerged in water and a thicker, greenish (or reddish) dorsal (upper) lobe. *Anabaena azollae* filaments are found in a cavity in the dorsal lobe of the fern's leaves, where unique circumstances promote a vegetative cell differentiation during leaf development and a high heterocyst frequency. For decades, rice paddies in China and other Asian nations have utilized *Azolla* and its nitrogen-fixing companion, *Anabaena*, as "green manure" to increase rice production through biofertilizer. A*zolla* supplies nitrogen, increases organic matter, enhances fertility of soil and shows tolerance against heavy metals. It grows very quickly in ponds and buckets, and makes an excellent fertilizer (green manure).

AIM: To study the symbiotic relationship through temporary tease mount of *Azolla* leaf.

REQUIREMENTS:
Material: *Azolla* sp.
Chemicals: Safranin, Glycerin, Distilled water
Instrument: Compound microscope, Dissecting microscope
Glassware: glass slide, watch glass, coverslip
Miscellaneous: forceps, needles, brush, filter paper, dropper

PROCEDURE:

1. Take an *Azolla* leaf and wash it with distilled water.
2. Keep washed leaf on microslide and add 1-2 drop of distilled water.
3. Tease the upper epidermis of dorsal lobe of *Azolla* leaf with the help of needle under dissecting microscope.
4. Add two drops of safranin to teased leaf and leave for 1-2 min.
5. Blot extra stain with blotting paper from edge of the material.
6. Add a few drops of distilled water to remove the extra stain, and blot the water.
7. Put a drop of glycerin to it.
8. Carefully cover it with a coverslip and avoid any air bubble.
9. Remove any excessive glycerin with a filter paper.
10. Observe the prepared temporary mount of the leaf under a compound microscope.

OBSERVATIONS:

Beaded filament with heterocyst, of *Anabaena azollae* will be observed in dorsal lobe of *Azolla* leaf under compound microscope.

PRECAUTIONS:

1. Teasing should be done gently and on upper epidermis of *Azolla* leaf only.
2. Over staining and under staining should be avoided.
3. Clean and dry glass slide and coverslip should be used.
4. Coverslip should be lowered down carefully avoiding trapping of air bubbles.

Post-activity Questions

1. What is Chemical Farming?
2. Why Say No to Chemical Farming?
3. Can we boost agricultural productivity in an eco-friendly manner? If yes, how?
4. What are Biofertilizers?
5. What are the various sources of Biofertilizers?
6. *Azolla-Anabaena* potential as a Biofertilizer?
7. Do we use same biofertilizer for all type of crops?
8. Five significances of organic farming.

DNA: CLOSE ENCOUNTERS

Dr. Madhu Bajaj and Dr. Renuka Agrawal

Pre-activity Questions

1. How does a prokaryotic cell differ from a eukaryotic cell?
2. What is a genome?
3. What is DNA?
4. Why is DNA fibrous in nature?
5. What are chromosomes?
6. How is DNA packaged into chromosomes?
7. Why is DNA Soluble in water?
8. Who proposed the currently accepted model for structure of DNA?
9. What is the composition of DNA?
10. What is the difference between nucleoside and nucleotide?
11. What are the different kinds of bonds present in DNA?
12. What are histones?
13. Why are histones basic in nature?

Aim: Isolation of Genomic DNA from Cauliflower by Spooling Method

Principle:

Structure of DNA: According to the model proposed by James Watson and Francis Crick, deoxyribonucleic acid (DNA) is a macromolecule composed of two long chains of deoxyribonucleotides joined by phosphodiester bonds. Each deoxyribonucleotide contains a phosphate group, a 5- carbon sugar 2-deoxyribose, and a nitrogen containing base. The complex of nitrogenous base and deoxyribose sugar is known as a nucleoside. The base is linked to the sugar by an N-glycosidic bond. There are four bases: two purines namely adenine and guanine; and two pyrimidines viz. thymine and cytosine. The two chains have distinct polarity and are wound around each other in an anti-parallel manner forming a double helix. In

the helix the strands are held together by hydrogen bonding between the bases. Adenine pairs with thymine through two hydrogen bonds and guanine pairs with cytosine by means of three hydrogen bonds. DNA contains the genetic information for the structure and function of living organisms which is organized into hereditary units called genes.

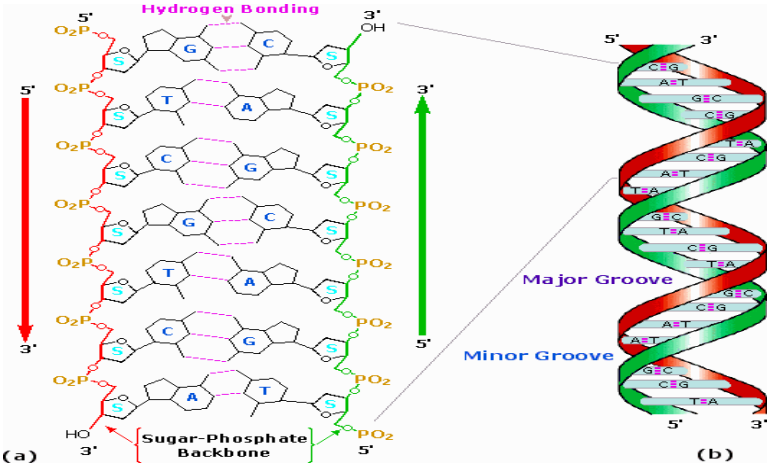


Figure 1. DNA structure: (a) sugar-phosphate backbone and anti-parallel nature of the two strands of DNA and (b) DNA double helix.
(Reference: https://www2.chemistry.msu.edu/faculty/reusch/virttxtjml/nucacids.htm)

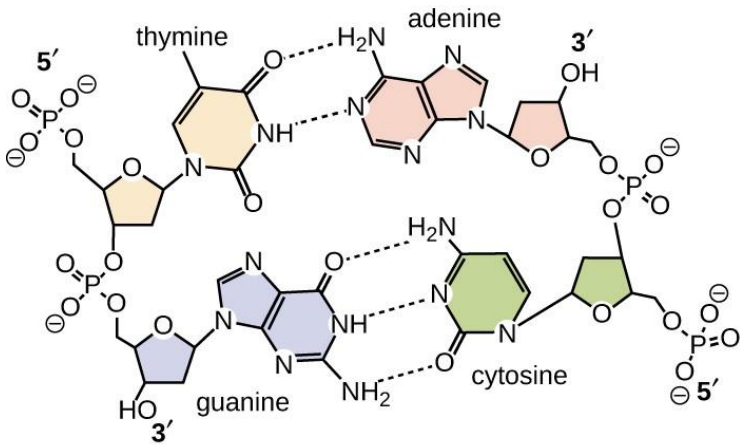


Figure 2. Hydrogen bonds form between complementary nitrogenous bases in the interior of DNA (Reference: https://courses.lumenlearning.com/suny-microbiology/chapter/structure-and-function-of-dna/).

Packaging of DNA into chromosomes: Each DNA molecule may be several cm long and is packed in tiny structure called chromosomes that are about 20,000 times smaller than the length

of the DNA molecules. Such an extremely small packaging of the DNA becomes possible due to supercoiling of DNA. In prokaryotes, the cell's entire genetic information is stored in a single circular chromosome whereas in eukaryotes it is organized into several linear chromosomes which are contained within a double membrane-bound nucleus. Further, in eukaryotes the negatively charged DNA interacts by means of ionic bonds with positively charged proteins known as histones to form a complex called chromatin. Histones are basic in nature because they are rich in positively charged amino acids lysine and arginine. A complete set of chromosomes (hence, of genes) inherited as a unit from one parent is called the genome.

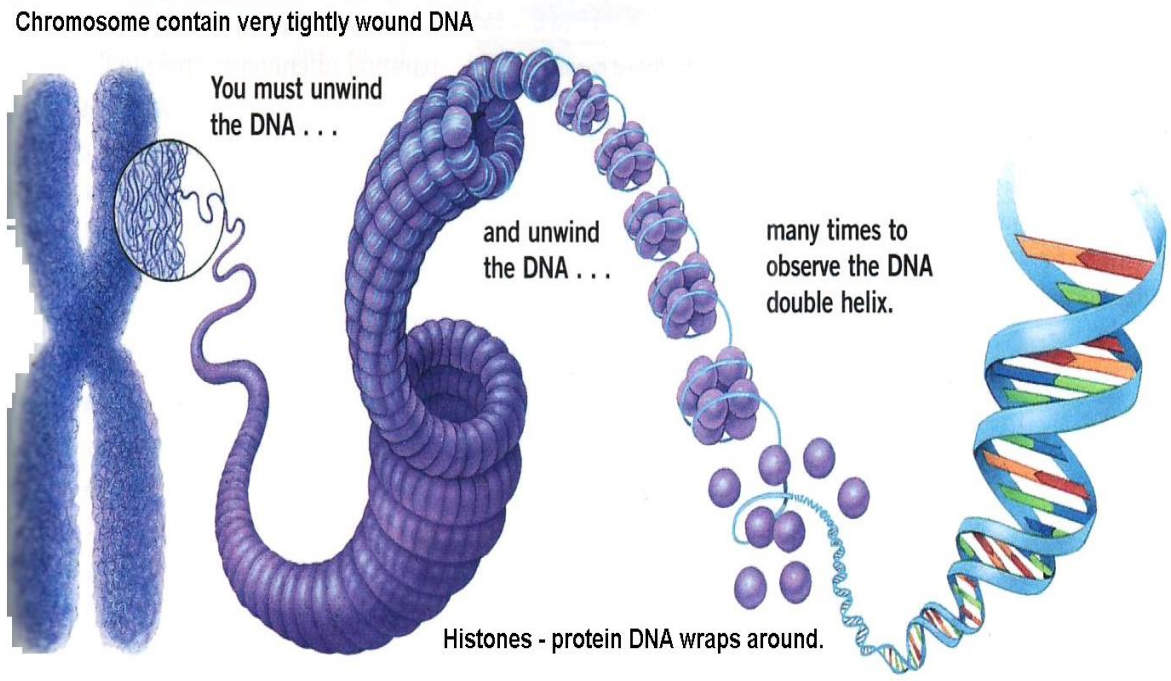


Figure 3. Packaging of DNA in eukaryotic chromosome.
(Reference: https://golifescience.com/structure-of-chromosome/)

Extraction of DNA: DNA can be easily extracted from tissues by using (1) a detergent to disrupt cell membrane and also to denature proteins (2) sodium chloride to break the ionic interactions between DNA and histones and (3) ethanol to dehydrate DNA from aqueous environment. DNA is soluble because of hydrogen bonding between water molecules and phosphate groups. The tissue is homogenized to separate the cells and break cell walls. The plasma membrane and nuclear membrane get disrupted by the detergent and chromatin is released. DNA can be isolated from the chromatin by using high salt concentration to break the electrostatic interaction between DNA and histones. Finally, the DNA can be precipitated from the solution by adding chilled ethanol; the lower temperature reduces the solubility of DNA,

and the alcohol with its low dielectric constant allows the sodium cations to associate with negatively charged phosphate groups resulting in breakage of bonds between DNA and water.

Requirements:

Plant material: One small cauliflower

Glassware and apparatus: Beakers (250 ml and 100 ml), measuring cylinders, test tubes, Pasteur pipettes, glass rod, hooked Pasteur pipette, Water bath at 60°C, chilled pestle and mortar, weighing balance

Chemicals: Sodium chloride, liquid detergent, chilled absolute ethanol

Miscellaneous: Ice bucket with crushed ice, cheese cloth (~ 25 cm), shaving blade or scalpel, butter paper, spatula, distilled water (DW)

Procedure:

- Remove about 3-4 mm thin shavings from the surface of a fresh and healthy cauliflower.
- Take 10 g of shavings in a 100 ml beaker. Add 30 ml of 5% liquid detergent solution and incubate in a water bath maintained at 60°C for 20 min.
- Transfer the contents to a pre-chilled mortar and place it in an ice bucket. Gently homogenize with the help of a pre-chilled pestle.
- Filter the slurry through four layers of cheese cloth into a 100 ml beaker. Let the solution drip for a while. If necessary, squeeze the cheese cloth but do not let any solid piece drop in the filtrate.
- Add 3 g NaCl to the filtrate and gently stir with a glass rod to dissolve the salt.
- Transfer the suspension to a test tube and keep it in the ice bucket for 5-10 min.
- Add 50 ml of ice cold ethanol down the sides of the test tube to form a layer. Observe DNA appearing as a viscous cloud in the test tube.
- Insert a hooked Pasteur pipette into the cloud and spool out the DNA fibres.
- Air dry the DNA and dissolve in DW for further study.

Expected Results: A viscous white cloud appears in the test tube that contains DNA as well as RNA. In order to get purified preparation of DNA the enzyme ribonuclease (RNase) needs to be added to digest the RNA.

Precautions:

- Pre-chilled mortar and pestle should be used.
- NaCl should be dissolved gently as DNA is prone to shearing.
- Filtration must be done carefully to avoid any piece of cauliflower.
- Pre-chilled ethanol should be used.
- Ethanol should be poured slowly and carefully along the sides of the test tube.

Post-activity Questions

1. Why is cauliflower used for the extraction of DNA?
2. Which other plant materials can be used instead of cauliflower?
3. Why must gloves be worn throughout the experiment?
4. What is the role of detergent in this experiment?
5. Why are the instruments and glassware autoclaved?
6. Why is the tissue incubated at 60^{o}C ?
7. What is the need for tissue homogenization?
8. What is achieved by filtration of the slurry?
9. Why is NaCl added to the filtrate?
10. Why should vigorous stirring of the suspension be avoided?
11. Why is the suspension maintained at low temperature?
12. Why is ice cold ethanol added to the suspension?
13. What is dielectric constant?
14. Can the DNA be spooled out by an ordinary glass rod?
15. How can RNA be removed from the extract?