

PW01: Observation of *Rhizobium* bacteria

Rhizobium bacteria establish a symbiotic relationship with leguminous plants, such as clover, by infecting their roots and forming nodules. Inside these nodules, the bacteria convert atmospheric nitrogen (N_2) into a form that the plant can use for growth (ammonium). This process, known as nitrogen fixation, is facilitated by the enzyme nitrogenase, which is active in the bacteroid form of the bacteria.

I. Objective:

- To observe *Rhizobium* bacteria within the root nodules of clover.
- To identify the bacteroid forms of *Rhizobium*, which are responsible for nitrogen fixation.

II. Materials and reagents

- Scalpel for cutting and crushing the root nodules
- Microscope slides and coverslips
- Bleach (sodium hypochlorite solution)
- Alcohol
- Methylene blue

II. Procedure

1. Collection of nodules: collect several root nodules from the clover plant.
2. Cleaning: perform a sequential wash of the root nodules:
 - First, wash them in alcohol to remove any contaminants.
 - Then, disinfect the nodules in a diluted bleach solution for a short period
 - Finally, rinse the nodules thoroughly in distilled water to remove any traces of bleach and alcohol.
3. Sample preparation:
 - Cut and crush the cleaned root nodule using a scalpel to release the *Rhizobium* bacteria inside.
 - Place the crushed nodule in a small amount of methylene blue for 2 to 3 minutes.
 - Add one or two drops of distilled water to dilute the methylene blue .
 - Place a coverslip on the sample .
4. Microscopic Observation: Begin by observing the slide at 10x magnification to locate the nodules then increase to 40x magnification to examine the bacteroid forms of *Rhizobium* inside the nodules.