**Unit 2 Non-Symbiotic Nitrogen Fixers**

**Free living Azotobacter- free isolation, characteristics, mass inoculums, production and field application.**

*Azotobacter*for the first time isolated by Beijernick and described *Azotobacter chroococcum* and *A. agilis. Azotobacter* are free living nitrogen fixing bacteria which reside in the soil and rhizosphere and fixes nitrogen in association with the other hosts. *A. chroococcum* and *A. agilis* is a water – borne species.

1. **Isolation of *Azotobacter***

* The species of *Azotobacter* are isolated by soil dilution plate technique on the nitrogen free medium (Jenson’s medium)
* After 3 days incubation, flat soft, milky, and mucoid colonies of *Azotobacter* grow on agar surface.

1. **Characteristic of *Azotobacter***

* ***Azotobacter*** is gram negative, rod shaped, aerobic bacteria. Size and shapes vary with each type of species.
* Each cell consists of peritichous flagella but motility of different species varies
* *Azotobacter* forms an insoluble black brown pigment containing melanin due to its oxidation by tyrosinase. The optimum environmental condition for Azotobacter: temperature 25-30°C, high humidity, aeration, pH 7.2-7.6, high salt concentration. Etc.
* *Azotobacter* mayproduce cyst resting structure.

1. **Mass production of inoculums**

* The starter culture of *Azotobacter* is prepared by transferring its loopful **Jenson’s medium** in culture flask. On this, it proliferates under aerobic condition when the flask are incubated at 30°C on shaker incubator.
* After 3 days incubation , the cells are harvested from jenson’s medium and filled in fermentor, by maintaining sufficient aerobic conditions, temperarure 30°C for a few days.
* At certain intervals, broth is tested for its purity and cell number. The broth is harvested when cell number reaches to 109 ml.

Suitable carrier( charcoal, farmland manure, peat, lignite etc) is dried and powdered passing through sieve. Calcium carbonate powdered added to neutralize the carrier followed by autoclaving. The harvested broth is poured over the carrier in such a way that 40% moisture is maintained. The inoculants is mixed and curing is done for a week, then carrier-based inoculants is packed in polythene bags so that it can be stored and sent in market for sale.

1. **Field application of *Azotobacter* inoculants**

*Azotobacter* inoculant is applied in the field for various crops. However, it is applied in field as given below-

* **Foliar application-** foliar application of *Azotobacter* biofertilizer individually or in combination with half of normal dose of chemical nitrogen fertilizer, increased the leaf production and leaf quantity in mulberry.
* **Seed treatment-**slurry of *Azotobacter* inoculants is prepared by mixing with water in a container. Seeds to be sown in field are soaked in slurry (2kg inoculants per hectare overnight). Then seeds are sown in field.
* **Seedling treatment –** slurry of *Azotobacter* inoculants is prepeared by mixing 1Kg of inoculants with 40 litres of water. The roots of transplanted seedlings are dipped in slurry for 15-20 minutes. Then seedlings are transplanted in the field. The remaining slurry is spread in the field.
* **Top dressing-** carrier- based inoculants is mixed with Farmland manure and soil in the ratio of 3:25:25 (w/w). this mixture is top dressed through out field especially young seedlings of rice.

**Positive effects of foliar application such as plant growth, plant biomass and yield are recorded.**