Hello students, welcome to the E – Learning session

Title of the E-learning unit is Free living nitrogen fixing microbes,

Module name: *Azotobacter* as biofertilizer.

I'm Harshala Gad, Assistant Professor.
St. Xavier's College Mapusa - Goa

Outline of this module

Identification of *Azotobacter*
Isolation of *Azotobacter*,
Mass multiplication of a *Azotobacter*
Production of carrier based inoculants
Techniques of field application and crop response,

The learning outcome of this module

To identify *Azotobacter*
You will learn the methods of isolation.
Moss multiplication of *Azotobacter*
Methodology of producing carrier based inoculants
Techniques of field application and crop response.

Azotobacter is a genus of usually motile, oval or spherical aerobic free living nitrogen fixing bacterium.

It resides in soil and rhizosphere and fixes nitrogen in association with hosts.

Azotobacter includes six species viz., *Azotobacter beijerinckii*, *A*. *paspali*, *A*. *chroococcum*, *A*. *hapalophils*, *A*. *vinelandii* and *A*. *miscellus*.

Azotobacter is a gram-negative, rod shaped, aerobic bacterium the cell dimension varies from 2-7 x 1-2.75µm size and shape of each cell vary with the type of species.

Cells show varying morphology, each cell consists of peritrichous flagella.

One of the unique features of *Azotobacter* is that it forms an insoluble black-brown pigment containing melanin due to oxidation by enzyme tyrosinase.

The optimum environmental conditions for its occurrence are: it can thrive in a temperature range of 25-30°C, it can tolerate high humidity, it requires aeration, and can occur in pH range of 7.2-7.6, can also tolerate high salt concentration.

.

In order to isolate *Azotobacter*, one needs species of *Azotobacter* which can be isolated by soil dilution plate technique.

10 grams rhizosphere soil is mixed well with 100 ml of distilled water and left undisturbed for some time to have clear suspension.

The suspension is serially diluted and inoculated into Petri dishes on nitrogen-free mannitol agar/Jensen's medium and incubated for 48 h at 30°C.

After 3 days incubation, one can observe flat soft, milky and mucoid colonies of *Azotobacter* grow on the surface of the medium.

Once pure culture of *Azotobacter* obtained one can proceed for mass multiplication

Azotobacter is transferred to a flask containing sterile Jensen's medium and incubated on a rotary shaker or batch fermentor for four days at 30°C.

The pure cells of *Azotobacter* developed in broth acts as starter culture.

In 1 litre of starter culture, about 100 litres of medium is transferred to sterilized Jensen's medium in a bioreactor at 30°C and continuous agitation for aeration.

When inoculum density reaches to 108 -109 cell/ml broth, it should be harvested to prepare carrier based inoculant.

Once the mass culturing of *Azotobacter* is achieved one can proceed for production of carrier based inoculants.

For production of carrier based inoculants suitable carrier such as peat charcoal, farm yard manure is dried and powdered passing through a sieve these carriers can be used alone or in combination.

Calcium carbonate powder is added to the carrier to neutralize. And it is sterilized in an autoclave.

The harvested broth containing *Azotobacter* is poured over the carrier in such a way that 40% moisture is maintained.

The inoculum is mixed and curing is done for a week.
The carrier-based inoculant is packed in polythene bags so that it can be stored for further use.

Coming to the application of *Azotobacter* in field

Azotobacter inoculants can be applied in field or to crops in falling methods:

Seed treatment, Seedling treatment pouring, pouring of slurry or Top dressing

Coming to the first one that is

Seed treatment: Slurry of *Azotobacter* inoculant is prepared by mixing with water. Seeds to be sown in field are soaked in the slurry overnight and then seeds are sown in field in the morning. The remaining slurry is directly sprayed in the field.

Seedling treatment: This method is applicable for transplantation crop. Roots of seedling to be transplanted are kept in slurry of carriers inoculants for 10 to 30 minutes. Seedlings are transplanted immediately.

Pouring of slurry: Carrier based inoculant is diluted in water. Small amount of slurry is poured near the root zone. Slurry may also be mixed with farmyard manual and administer near root zone.

Top dressing: Carrier-based inoculants is diluted in water at the rate of 2 kg/hectare and mixed with farm yard manure at the rate of 20-25 kg/ha.

After transplantation of cereal crops such as rice and wheat, it is broadcasted by top dressing.

Crop response after field application of *Azotobacter* inoculants at the Indian Agricultural Research Institute in New Delhi, field trials were conducted on different types of crop plants such as maize, sorghum, cotton, vegetable crops, wheat, rice etc at different places which resulted in better response of crop plant to *Azotobacter* inoculants.

The beneficial effect of *Azotobacter* is due to N_2 fixation and synthesis of growth promoting substances.

Azotobacter is also reported to synthesize some antagonistic substances which suppresses the growth of *Fusarium*, *Aspergillus* and *Alternaria* and reduces crop disease caused by these soil-borne pathogens.

A significant increase is observed in dry matter of sorghum and maize with combined treatment of *Azotobacter chroococcum + Azospirillum brasilense*.

These are the references for further reading,

Thank you.