



Azospirillum and *Azotobacter*

1. What is *Azospirillum*?

Azospirillum is a Gram-negative, microaerophilic, non-fermentative and nitrogen-fixing, motile, vibrioid bacteria belongs to the family of Rhodospirillaceae. The carbon sources that provide energy are malate, succinate, lactate and pyruvate. It grows moderately on galactose or acetate containing medium and poorly on glucose and citrate containing medium.

2. What is the isolation process of *Azospirillum*?

Azospirillum can be isolated from plant roots as well as soil sample. After collection of host plant root system, washed it with running tap water and cut into small pieces of about 0.5 cm. Root pieces are sterilized with 0.1% HgCl₂ solution for one minute. Then these are serially washed with sterile distilled water. Root pieces are placed on semi-solid agar medium containing sodium malate in screw-capped tubes. Then inoculated tubes are incubated at 28-30°C for 2 days. Just 1-2 cm below from the surface of medium, white pellicles of *Azospirillum* can be absorbed. Thereafter, the nitrogen-free malate medium becomes blue in color which shows the presence of *Azospirillum*.

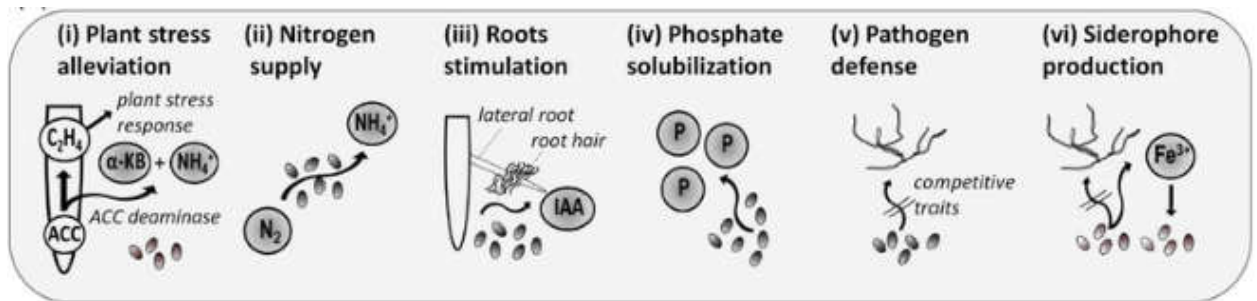
3. What do you mean by mass multiplication of *Azospirillum*?

Mass cultivation of *Azospirillum* is prepared by transferring its loopful colony to ammonium chloride containing Okon's medium culture flask. Inoculants proliferates profusely on this medium under aerobic condition when the flasks are incubated on a rotary shaker at 35°C. After 3 days the cells can be harvested for inoculation of Okon's medium filled in a fermentor and sufficient aerobic conditions are maintained in fermentor for a few days. At certain intervals, broth is tested for its purity and cell number. *Azospirillum* inoculums is harvested when cell number reaches to 10⁹/ml.

4. Explain the preparation of carrier based inoculants for the *Azospirillum* inoculums.

Low cost and locally available carrier have been used for the preparation of *Azospirillum* containing bio-fertilizer. For example Farmyard Manure (FYM), FYM with charcoal, FYM with soil (sterilized soil and FYM in the ration of 1:3 is best for *Azospirillum*), peat etc. the bacterial cell can survive for the 6 month and restore about 10^6 cells/gm in the carrier material of FYM and soil.

5. Explain the associative effect of different microorganisms.



Following the colonization or association with plant roots and/or the rhizosphere, bacteria can promote plant growth through (i) the alleviation of plant stress through the reduction of ethylene levels; (ii) the increase of nitrogenous resources through N_2 fixation; (iii) the stimulation of root development by phyto-hormone production; (iv) P solubilization around roots; (v) the defense against pathogens via the presence of competitive traits such as antibiotic and siderophores production; (vi) Fe solubilization around roots via siderophores production. C_2H_4 (ethylene), ACC (ethylene precursor in plants), α -KB (α -ketobutyrate), NH_4^+ (ammonia), IAA (indole-3-acetic acid).

6. What is *Azotobacter*?

Azotobacter is free living, nitrogen fixing, heterotrophic bacterium that derives energy by degrading plant residues. They are found in slightly acidic (*A. beijerincki*) to neutral alkaline (*A. chroococcum*) soil.



7. What do you mean by crop response to *Azotobacter* inoculums after field application?

At the Indian Agricultural Research Institute (New Delhi), field trials were conducted on various crops such as maize, sorghum, cotton, vegetable crops, wheat, rice etc. at different region. Always better responses of crops to *Azotobacter* were recorded. For example some crop yield increased with *A. chroococcum* inoculants by 6.7-71.7% over the uninoculated control. The beneficial effects of *Azotobacter* are due to N₂ fixation (*Azotobacter* fixes 20-40 kg N/ha/annum), synthesis of growth promoting substances and antifungal antibiotics.

8. What is the classification of *Azotobacter*?

Classification of *Azotobacter*

Domain: Bacteria

Kingdom: Bacteria

Phylum: Proteobacteria

Class: Gammaproteobacteria

Order: Pseudomonadales

Family: Pseudomonadaceae/ Azotobacteraceae

Genus: *Azotobacter*

9. What are the important characteristics of *Azotobacter*?

- a. It is gram negative, rod shaped, aerobic bacterium.
- b. Size and shape of each cell varies with their species type. That's why cells show varying morphology.



COMPILED AND CIRCULATED BY BANGAMOTI HANSDA, ASSISTANT PROFESSOR,
DEPARTMENT OF BOTANY, NARAJOLE RAJ COLLEGE

- c. Each cell consists of peritrichous flagella but motility varies in different species.
- d. *Azotobacter* can form insoluble black brown pigment containing melanin due to its oxidation by tyrosinase.
- e. High humidity and high salt concentration are needed for the growth of *Azotobacter*.
- f. Optimum temperature (25-30°C) and optimum pH (7.2-7.6) are required for the growth of *Azotobacter*.
- g. Cell may produce resting structure known as **cyst**. Cysts differ from vegetative cell having spherical cell with contracted cytoplasm and double coloured thick wall.
- h. Encysted cells can overcome unfavorable environmental conditions such as desiccation and exposure of UV light.
- i. Cyst can germinate to liberate cell through breaking cyst coat.

10. Write short note on maintenance and mass multiplication of *Azotobacter*?

Azotobacter inoculants transferred to a flask containing sterile Jensen's medium and the flask is incubated on a rotary shaker for a few days at 30°C. This broth culture acts as starter culture which may be used for mass production of inoculants. When the inoculum density has reached to 10^8 - 10^9 cell/ml broth, it should be harvested so that carrier based inoculants should be prepared.

Suitable carrier material is prepared and calcium carbonate powder is added to neutralize the carrier material followed by sterilisation by autoclaving. The harvested inoculants broth is mixed with carrier and curing is done for a week in such a way that 40%

moisture is maintained. Then carrier based inoculants are packed in polythene bags so that it can be stored or sent to market for sale.

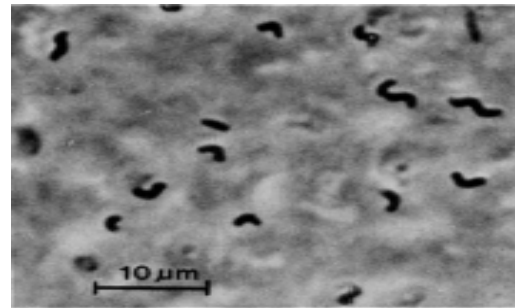


Fig 1: Microscopic view of *Azotobacter* sp. Fig 2: Microscopic view of *Azospirillum* sp.

References:

1. Dubey R.C., 2005 A Text book of Biotechnology S.Chand & Co, New Delhi.
2. <https://en.wikipedia.org/wiki/Azospirillum#:~:text=Azospirillum%20is%20a%20Gram%2Dnegative,bacteria%20can%20promote%20plant%20growth.>
3. https://www.researchgate.net/figure/a-The-different-types-of-association-between-plant-roots-and-beneficial-soil-bacteria_fig1_284094196
4. <https://sites.google.com/site/azotobacterwebsite/classification>
5. <https://www.quora.com/What-are-some-examples-of-non-symbiotic-bacteria>
6. https://pdfs.semanticscholar.org/6574/3bb98d2e59b112c8b670f49d9f8f7f339379.pdf?_ga=2.209630418.1176346009.1602834293-1975954642.1571808367

(All the information is collected from above references and will be used only for teaching and learning purposes)