



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

Rhizobium

BY

BANGAMOTI HANSDA

ASSISTANT PROFESSOR

DEPARTMENT OF BOTANY

NARAJOLE RAJ COLLEGE



1. What is *Rhizobium*?

It is gram negative soil bacteria that can convert atmospheric nitrogen into ammonia within root nodule of plants using the nitrogenase enzyme. They are non sporing bacteria.

2. What are the importances of *Rhizobium*?

Rhizobium helps in fixing nitrogen in leguminous plants by symbiotic association within the root nodule.

Nitrogen fixation also increases soil fertility.

It also act as biocontrol agents with suppressive effect against fungal pathogens.

3. Write some species name of *Rhizobium* and their host plant.

Rhizobium leguminosarum; host plant (*Pisum sativum*)

Rhizobium phaseoli; host plant (*Phaseolus vulgaris*)

Rhizobium trifolii; host plant (*Trifolium sp.*)

Rhizobium meliloti; host plant (*Melilotus sp.*)

4. What is the association between *Rhizobium* and leguminous plants known as?

The relationship between *Rhizobium* and leguminous plants is known as Symbiotic relationship.

In this relationship, both the organisms are benefitted from each other. *Rhizobium* takes shelter in the root nodule and receives nutrition in the form of organic acids. On the other hand, bacteria can fix nitrogen and make it available to the plants.

5. How do you Isolate *Rhizobium* from leguminous plant roots?

Isolation process is based on three major steps

a. Collection of root nodule:

Carefully uproot the leguminous plant and wash its root under running tap water to remove the soil dirt or soil particle. The colour of root nodule varies from brown to depending on the presence of pigmentation. It is always better to pick without break the root nodule.

b. Surface sterilization of root nodules:

After the washing of root nodules, the surface of these root nodules must be sterilized by sterilizing agent 0.1% mercuric chloride or 3-5% hydrogen peroxide fore 4-5 mins. Then wash it repeatedly with distilled water. After that they are again washing with 70% ethanol and again wash with distilled water.



c. Serial dilution method to isolate bacteria:

At first 1gm root nodules is crushed by motor pestle and mixed with 10 ml distilled water to get nodular extract suspension. Then 10 fold dilutions is prepared by taking 1ml from suspension of root nodule and added to 9ml distilled water fore making the 10^{-2} dilution. Similarly repeat the process up to 10^{-8} or 10^{-9} .

YEMA (Yeast Extract Mannitol Agar) plates are prepared and inoculated with 0.1ml of serial diluted nodule extract by spread plate method. Then incubate it at 28-30 °c fore 3-4 days. Bacterial colonies are appeared on the surface of the culture media.

6. What are the constituents of YEMA?

Yeast extract 1.0 gm, Mannitol 10.0 gm, $K_2 HPO_4$ 0.5gm, $MgSO_4 \cdot 7H_2 O$ 0.2gm, NaCl 0.1gm, Agar 20gm, distilled water 1 litre, pH 0.5-7.

7. What are the colony characters of *Rhizobium* bacteria on culture media?

Colonie are white, translucent, glistening and elevated with entire margin

8. How can you distinguish *Rhizobium* from *Agrobacterium* from culture media?

Rhizobium and *Agrobacterium* both can grow on YEMA medium. These two genera can be distinguished by following tests-

Glucose Peptone Agar test: Replica petri plates containing Glucose Peptone Agar (GPA) medium are prepared and inoculated with bacterial colonies. *Agrobacterium* can grow on this media but *Rhizobium* cannot grow on Glucose peptone Agar media.

Salt tolerance test: *Rhizobium* cannot grow on YEMA plate containing sodium chloride (NaCl) but *Agrobacterium* is able to grow on such medium.

Lactose test: Lactose agar media are inoculated by bacteria and incubated fore 4-10 days. Thereafter media are poured with Benedict's reagent. Then the appearance of yellow colour shows the presence of *Agrobacterium* due to the utilization of lactose. But *Rhizobium* cannot utilize lactose from agar media containing lactose.

9. What are the carrier materials for inoculants?

Various type of carrier materials are used such as peat, lignite, farmyard manure and charcoal powder.

10. What do you mean by carrier material?

It should be cheaper in cost and easily available.

It has no toxic chemicals and can be sterilized by autoclave.



Water holding capacity of carrier material must be more than 50%.

it should be contain high organic matter and can be processed very easily.

11. Write short note on Mass culture of Rhizobium

Fore the mass cultivation of rhizobium, sterilize the growth media and inoculate the media with inoculums of mother culture. Then incubate the growth media fore 3-4 days at 30°C in the incubator. Taste the culture fore its purity.

Transfer the culture to a large fermenter fore 4-9 days fore the good bacterial growth. The pH is adjusted to 6.5-7.0 by the addition of KOH or H₂ SO₄ solution. As per on the demand bacterial culture may be carried out in large fermentor or in small flasks.

12. What is actinorhizal symbiosis?

Actinorhizal symbiosis is a symbiotic relationship between nitrogen fixing actinobacteria *Frankia* and Actinorhizal plants, results in formation of root nodules.

13. Write some example of actinorhizal hosts that can associate with *Frankia*.

Eight angiosperm families form actinorhizal symbiotic relationship with the actinomycete bacterium *Frankia*. e.g. Datisceae – *Datisca sp.*; Casuarinaceae – *Casuarina sp.*

14. What is the importance of *Frankia*?

It plays an important role in nitrogen cycling and protect from O₂ poisoning.

It can fix nitrogen as free living bacteria and forms root nodule in actinorhazal plants.

15. Give characteristic features of *Frankia*.

Frankia is a gram negative and gram variable bacteria belong to Frankiaceae family.

They can grow in hyphal form in aerobic conditions. They form spherical vesicles at the end of hyphae or short side hyphae fore the fixation of nitrogen.

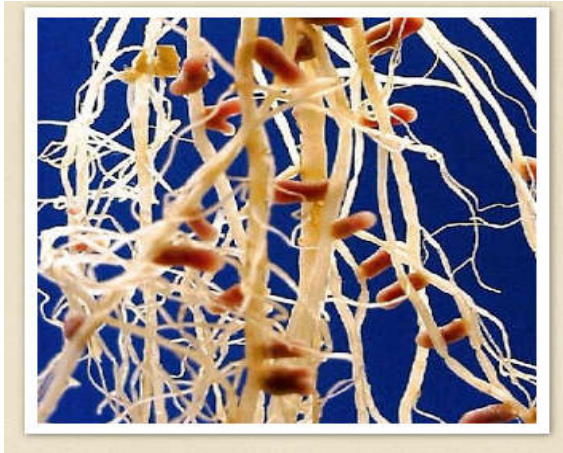


Fig1: *Rhizobia* bacteria on the roots of legumes

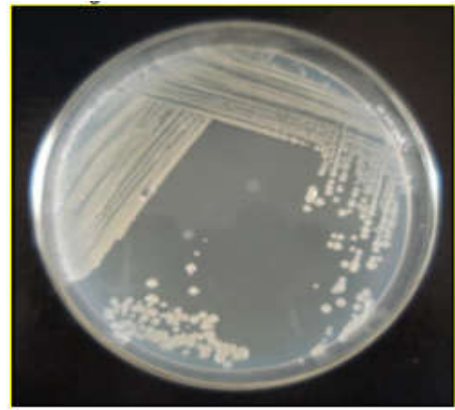


Fig2: *Rhizobium leguminosarum*
on YEMA plate

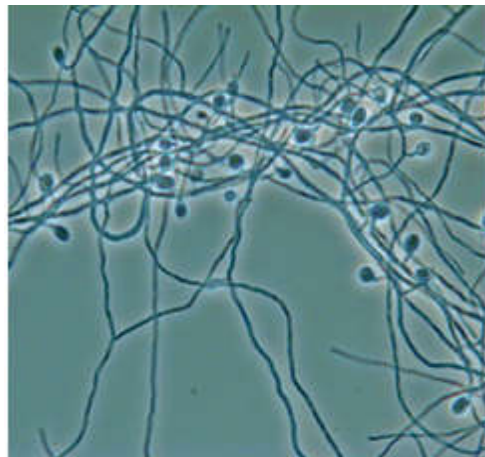


Fig3: Strain of *Frankia sp.*

References:

1. <http://onlinescienceallthetime.blogspot.com/2010/08/rhizobium-bacteria.html>
2. Vishal Kumar Deshwal and Abhishek Chaubey 2014. Isolation and Characterization of *Rhizobium leguminosarum* from Root nodule of *Pisum sativum* L. Volume2, Issue8.
3. https://genome.jgi.doe.gov/portal/fra_c/fra_c.home.html

(All the figures are collected from the above references and will be used fore teaching and learning purposes)