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Center for Smart Agriculture

Isolation, purification & mass multiplication of Azospirillum

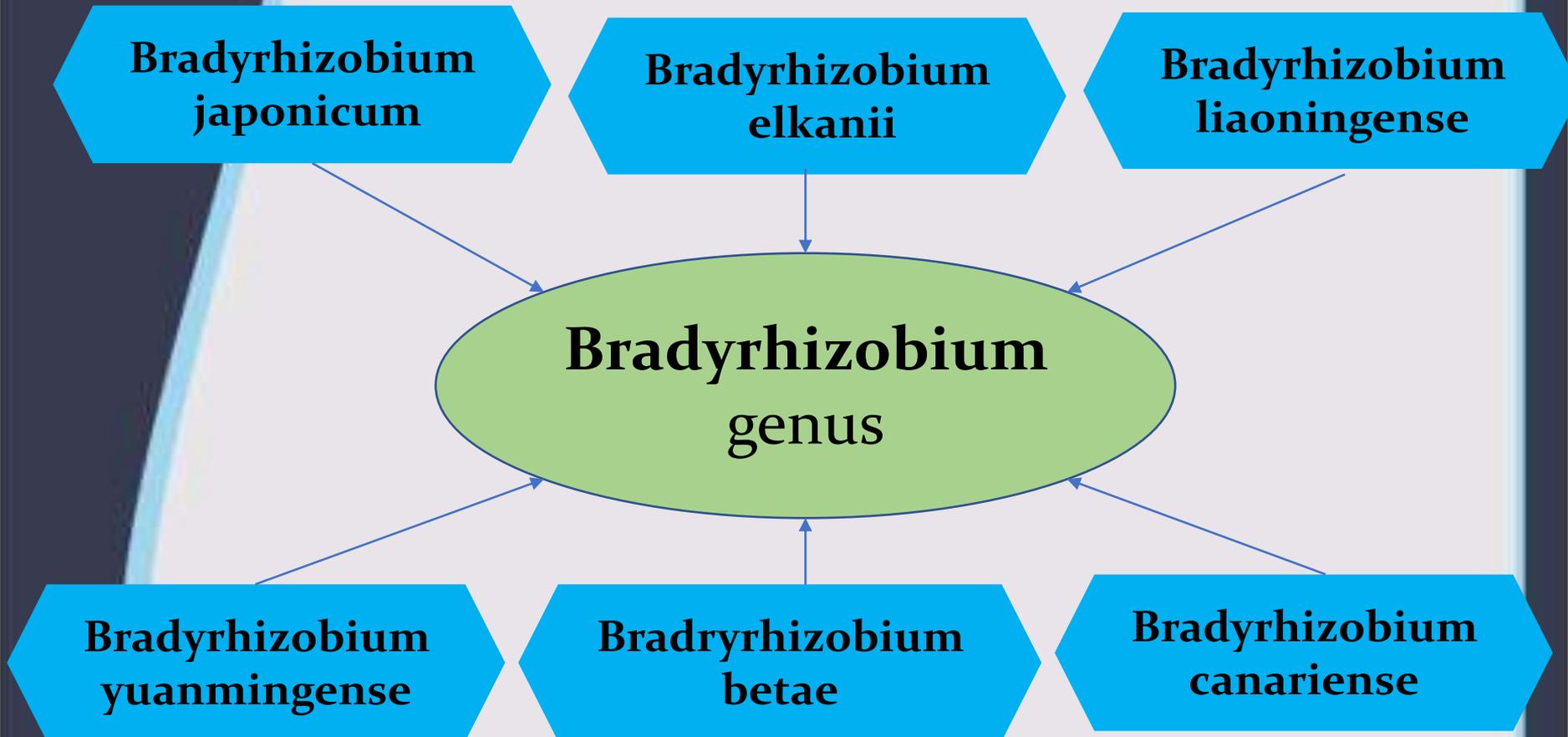


Module 1:
Session 1:



Rhizobium Inoculant

Bradyrhizobium strains are slow growing, gram negative, soil bacteria.





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Isolation of Rhizobium Strains

- Collection and preservation of root nodules in field trips
- Equipment for collection and preservation of root nodules
- Sampling vials with desiccated silica gels



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Consideration points before sampling

- a) Site identification
- b) Host plants identification
- c) Soil identification:



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Collection and preservation of root nodules

Excavate whole plants



Remove soil around root nodules



Store all nodules from a single host plant in the same vial



kept vials in a refrigerator at 4 °C



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Isolation of bradyrhizobia from dried root nodules

(1st day)

- Wash dried root nodules under tap water to remove soil contamination and transfer to sterile water.
- Keep the root nodules in sterile water in a refrigerator at 4 °C overnight to absorb water.



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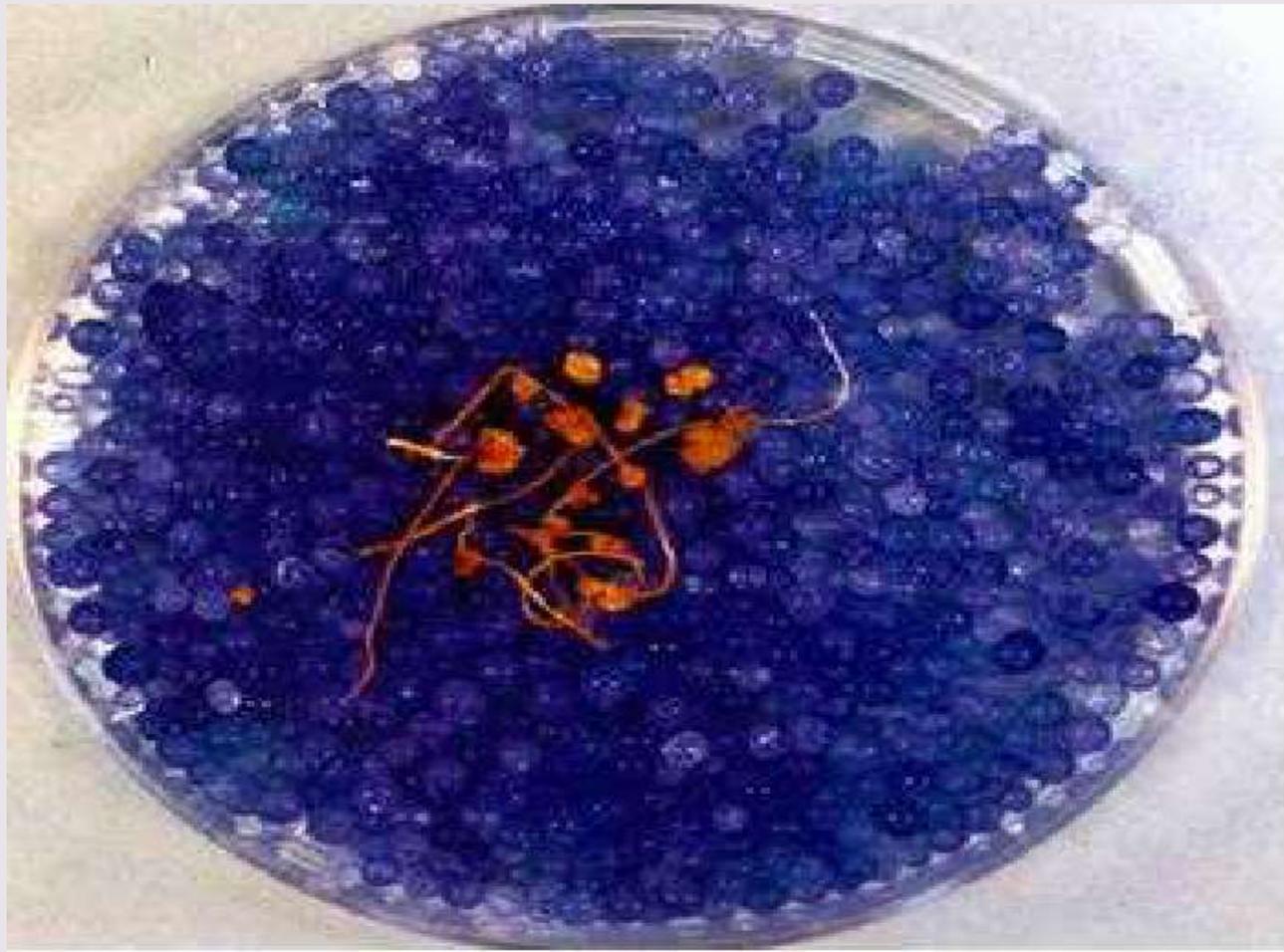


Fig 1: Dried root nodule in blue silica gel



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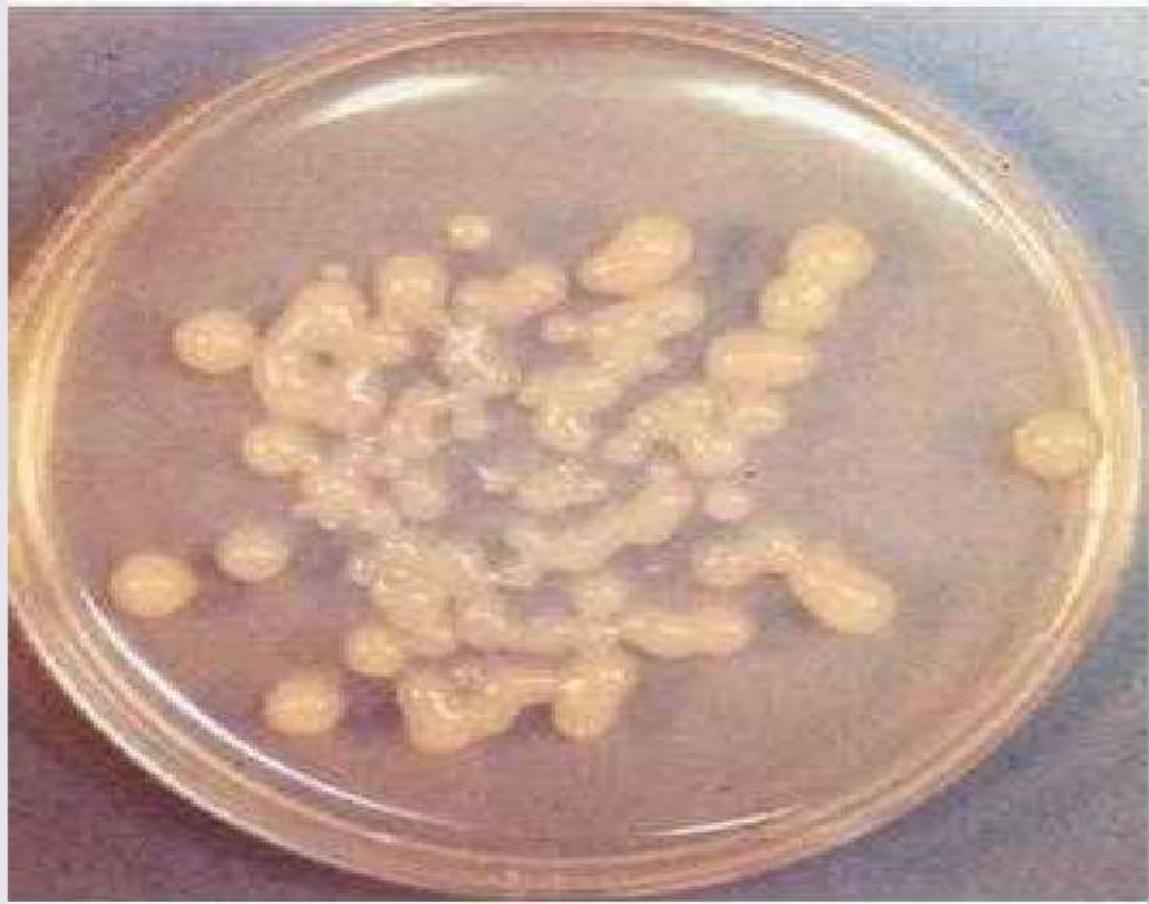


Fig 1.1: Bradyrhizobium colonies isolated from a dried root nodule originated in Wild Vigna plant



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(2nd day)

- The root nodules are surface sterilized by immersion in 70 % ethanol for 30s, and in a 3 % sodium hypochlorite solution for 3 min.
- The root nodules are subsequently washed at least five times with sterile water.
- Each root nodule is crushed in a microfuge tube with 100 μ l of 15 % glycerol solution.



(2nd day)

- Ten μl of turbid suspension with 15 % glycerol solution is streaked onto the surface of a yeast extract mannitol agar (YMA) medium (Somasegaran and Hoben 1994).
- The turbid suspensions with 15 % glycerol solution in a microfuge tube keep in freezer at $-30\text{ }^{\circ}\text{C}$ until isolations of bradyrhizobia are complete.
- If first isolations do not succeed, the glycerol solutions are restreaked onto fresh plate to obtain pure cultures.



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(2 weeks later)

- The plates are incubated at 28 °C for 2 weeks in an inverted position. Well-separated single colonies are restreaked onto fresh plate to obtain pure cultures.



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Thank you...