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Isolation, Phenotypic Characterization and Authentication Protocol of Root Nodule Bacteria (Rhizobia) in Promoting Sustainable Agricultural Productivity: A Review

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Abstract

The capacity of indigenous rhizobia to nodulate a specific legume crop effectively, or to act as barrier to the successful establishment of inoculant strains, is critical to successful establishment and growth of legumes in the respected agroecological zone. Effective groups serve both as a guide to inoculant preparation and as a basis for predicting the need for inoculation in future perspective. This review included all aspects of rhizobial work basic protocols, starting from isolation and characterization of root nodule rhizobia from the laboratory to authentication of the symbiotic effectiveness test under sterile sand condition in the greenhouse. This review shows the process on how to isolate, characterize and evaluate the symbiotic effectiveness along with growth responses to varied physiological conditions of pH, temperature, antibiotics, heavy metals as well as wide range of carbon and nitrogen sources. The main goal of repeated works in this regard is to discover new and better strains for use in legume inoculants so as to improve agricultural productivitiy. This pursuit entails the collection of isolates, characterization, assessment of symbiotic capacity and comparison to strains currently included within inoculants. This was a very valuable review for researchers not only for accomplishing initial steps for getting new strains but dealing with improving soil fertility and supporting sustainable crop production with the minimum use of costly and environmentally unfavorable chemical fertilizers.

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Inoculant, Rhizobium, Identification, Isolation

Introduction

Soil and Nodule Sample Collection

Pink colored nodules and 3 kg of soil was collected from the roots and rhizosphere of healthy standing plant, respectively. The nodules collected and been placed in sealed vials containing a desiccant (Silica gel) covered with 1cm of cotton wool and brought to Wollo university Biology laboratory and kept at 4^0C till isolation of the rhizobia was conducted (Somasegaren and Hoben, 1994). The soil was placed in polythene plastic bags and brought to laboratory for further work.

Isolation of Rhizobia

Dehydrated or desiccated root nodules were immersed in sterile distilled water for overnight in labeled sets of petri-dishes (Vincent, 1970). The imbibed nodules were surface sterilized according to Vincent (1970). The nodules were first be subjected to 70% ethanol for 10 seconds and then to 5 % (v/v) solution of sodium

hypochlorite (NaoCl) for 4 minutes. The surface sterilized nodules were then be rinsed in five changes of sterile distilled water to completely rinsed the sterilizing chemicals. The surface sterilized nodules were transferred into sterile Petri-dishes and crushed with alcohol flamed sterile glass rod in a drop of normal saline solution (0.85% NaCl) inside a laminar air flow hood.

A loopful of suspensions or crushed nodule saps was streaked across the surface of Yeast Extract Mannitol Agar (YEMA) plates containing 25 ppm of Congo red (YEMA-CR) and was incubated at 28 ± 2^0C from 3-5 days. Isolates that didn't absorb Congo red was selected for further purification. YEMA contains the following composition in amount g/l: Mannitol (10), K_2HP0_4 (0.5), $MgS0_4$. $7H_20$ (0.2), NaCl (0.1), Yeast Extract (0.5), Agar (15), Distilled Water (1000ml).The pH was adjusted at 7±0.1 and Autoclaved at 121^0_C for 15 minutes.

Purification of Isolates

Different single and separate colonies were picked from YEMA plates with sterile inoculating loop and transferred into 6ml of sterilized YEM broth in test tubes. The test tubes were vortexed and placed on rotary shaker for 48 hrs at room temperature. After two days, a loop-full of culture suspensions from each test tube was taken and streaked on sterile YEMA plates and incubated at 28 ± 2 ^{0}C for 3-5 days (Somasegaren and Hoben, 1994). The purity and uniformity of the colony was confirmed through repeated re-streaking.

Designation of Rhizobial Isolates

All the purified rhizobial isolates were designated as WUCR (Wollo University Chickpea Rhizobia) with different numbers representing each isolates.

Presumptive Test

The growth of the rhizobial isolates were determined by growing them on Peptone Glucose Agar (PGA), Keto-Lactose Test, Gram staining and YEMA-CR medium according to (Somasegaren and Hoben, 2012). The isolates were incubated at 28 ± 2 ^{0}C for 3-5 days.

Growth on YEMA with Congo red

The characteristics of the rhizobial isolates to absorb Congo red were checked by growing the isolates on YEMA- CR (Vincent, 1970). The isolates were incubated at 28 ± 2 ^{0}C for 3-5 days. The isolates that absorbed and failed to absorb Congo red were recorded.

Growth on Glucose Peptone Agar (GPA) Medium

Glucose peptone agar medium with Bromo-thymol blue (indicator) was used for isolating pure rhizobial colonies. Heavy growth is indicative of contamination. Glucose peptone agar medium was prepared and bacterial isolates were streaked on plates and plates were incubated at 28^{o}c for 48 hours (Vincent, 1970). Isolates that was shown no growth or very poor growth on glucose peptone agar medium was taken as rhizobia. The media composition amount in g/l, peptic digest of animal tissue (20), Dextrose (10), Sodium chloride (5),Agar (15). Final pH adjusted 7.2±0.2.

Keto-Lactose Test

The rhizobial isolates were streaked on Keto-Lactose agar medium to tentatively proof they belong to rhizobial isolates. The plates were incubated at 28 ^{0}c for 48 hours. After 48 hrs, the plates were flooded with Benedict's reagent incubated at room temperature and the presence of ring around the colonies were checked (Holt, 1994). The composition in g/l: Yeast extract (10), Glucose (20), Calcium carbonate (20), Agar (15) and dH_2O (1000ml).

Gram Reaction of the Isolates

The rhizobia isolates were tested for determining their Gram reaction using the KOH method (Buck, 1982). A drop of 3% KOH was placed on a clean microscope slide and a loopful of rhizobia colony mixed with it properly for l min.

The mixture was lifted with inoculating loop about 1 cm from the slide and presence and absence of obvious stringiness (viscosity) was observed as Gram-negative and Gram-positive bacteria, respectively as the methods used by Mussa Adal *et al.*, (2018).

Colony and Growth Characteristics

The morphological characteristics of each isolates were determined according to (Lupwayi and Haque, 1994). A loopful of 48 hrs old grown broth culture from each isolate was inoculated onto YEMA and incubated at 28±2^0C for 3-5 days. After 5 days, colony diameter, colony morphology and colony texture and pigmentation were recorded (Martinez-Romero *et al.*, 1991).

Acid/Base Reaction

To determine the ability of the rhizobial isolates to produce acid or alkaline in YEMA medium containing Bromthymol Blue (BTB) (0.025w/v) was used. A loopful of the isolates from a 48 hrs old culture broth was streaked on the YEMA –BTB medium and incubated for 3-5 days so as to record the color changes as stated by Jordan (1984).

Mean Generation Time (MGT)

Bacterial growth was assessed on YEM broth in Erlenmeyer flask incubated in a Gyratory shaker at 130 rev. min^{-1}. Optical density was measured using Spectrophotometer (Jenway 6405 UV/vis.) at 600nm every 6hrs for 24hrs. Mean generation time or doubling time was calculated from the logarithmic phase (White, 1995; Somasegaran and Hoben, 1994).

g = [log2 (t)]/ [logX-logXo]

Where t is time elapsed, Xo is first OD reading and X is second OD reading in logarithmic phase

Greenhouse Authentication of Rhizobia

Sterilized sand was used to identify the definitive test of all the rhizobial isolates and then screened for infectivity and effectivity. Pots were washed and surfaced sterilized and autoclaved as indicated in Somasegaren and Hoben (1994). Chickpea variety called "Desi" was used for the greenhouse experiment. Gray undamaged seeds of uniform size were selected and surface sterilized and was rinsed in five changes of sterile water and placed to 0.75 (w/v) water agar plates. They were incubated at 28 ± 2^0C for 3 days. After three days, five germinated seeds were transferred into each surface sterilized pot for germination which was thin down into three later.

After 3 days, each seedling was inoculated with 1ml of 72 hrs YEMB grown culture. The experiment was statistically laid out with three replications using a complete random design in a greenhouse with a 12hr. photoperiod and an average of 28^0C and 15^0C day and night temperature. The experiment consisted of negative control, positive control and bacterial treated treatments. All the plants were fertilized with quarter strength of Broughton and Dilworth N Free medium (Table 3) per week as described in Somasegaren and Hoben (1994).

After preparing the stock solutions, 10 liters of full strength nitrogen-free plant nutrient solution was prepared by mixing 5 ml of each stock solution with 5 liters of distilled water in a container and further diluting it to 10 liters by adding another 5 liters of distilled water (Somasegaren and Hoben, 1994). The pH of the solution was adjusted to 6.8 with NaOH and HCl. Hundred ml of quarter-strength N-free nutrient solution was supplied to each pot once every week. All the pots were watered every two days.

Relative Symbiotic Effectiveness of the Rhizobial Isolates

Fifty days after planting, plants were uprooted to record shoot length, nodule number, nodule dry weight and shoot dry weight. The symbiotic effectiveness (SE) of the isolates was calculated using the formula:

$$\%\,SE = \frac{\text{Inoculated plants SDW}}{\text{N} - \text{Fertilized plants SDW}} \text{X100}$$

As described previous research (Molungoy, 2004)

Where, SDW = shoot dry weight, N= nitrogen, SE= symbiotic effectiveness. The rate of nitrogen fixing effectiveness was evaluated as: Highly effective ≥ 80%, Effective 50-79%, Lowly effective 35-49% and Infective <35%.

Physiological Properties of Rhizobial Isolates

Salt Tolerance

Salt tolerances of the isolates were cheeked by growing them on YEMA plates containing different concentration of salt (Gao*et al.*, 1994). YEMA was prepared with different concentrations of NaCl containing 0.5%, 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 11%, 12% and 13% of NaCl as indicated in Bernal and Graham (2001).

Growth at Different pH Test

The pH tolerance of the isolates were determined by growing them on YEMA at different pH values of 4, 4.5, 5, 5.5, 6, 8, 8.5, 9, 9.5 and 10 (Gao*et al.*, 1994). 100ml YEMA medium solution was prepared for each pH test and the pH of each was adjusted using 0.1N HCl or NaOH. Then 1.5g agar mixed with 100ml YEMA solution, autoclaved at 121°C for 15 min and YEMA plates were prepared with different pH concentration

(Somasegaran and Hoben, 1994). The plates were incubated at 28±2^{0}C for 3-5 days and absence and presence of growth was recorded.

Temperature tolerance test

YEMA media was used to investigate the growth response of rhizobial isolates to different temperature by inoculating and incubating the media at 5, 10, 15, 20, 35, 40, 45 and 50^{0}C (Lupwayi and Haque, 1994; Hungaria*et al.*, 2000). The temperature tolerance test above 45^{0}C was used broth media. After incubation period of 3-5 days absence and presence of growth was recorded.

Antibiotic tolerance test

The Rhizobia isolates were tested for antibiotic sensitivity by Kirby-Bauer disc diffusion method on YEMA agar media (Bauer *et al.*, 1966), and observed there activities of forming inhibition zone or not. But due to the lack of some antibiotic discs, powder also used. A loopful of bacterial isolates was inoculated on Muller Hinton Agar (MHA) amended with the required amounts of antibiotics, gentamycin, streptomycin, chloramphenicol and ampicillin adjusted media supplemented with a concentration of 2.5 µg ml^{-1}, 5 µg ml^{-1}, 10 µg ml^{-1} (Lupwayi and Hagqe, 1994; Mhamdi *et al.*, 1999). The plates were incubated at 28±2 ^{0}C for 3-5 days. Isolates were considered resistant when growth occurred and sensitive when no growth was detected.

Heavy metal tolerance

It was determined by growing cultures on solid media (YEMA) containing filter-sterilized heavy metal at concentrations (µg ml^{-1}) of Hg (HgC_{l2}) (5), Cr ($K_2Cr_2O_7$) (50), Mn ($MnCl_2$) (200), Ni ($NiCl_2$) (200), Pb ($Pb(CH_3COO)_2$) (50) and Zn ($ZnCl_2$) (100) according to Mohamed *et al.*, (2012) and monitoring growth of colonies after 3–5 days of incubation at 28± 2°C. Isolates were considered resistant when growth occurred and sensitive when no growth was detected.

Carbohydrate utilization

The carbohydrate utilization test of the rhizobial isolates was evaluated using 11 carbon sources: D-fructose, mannitol, sucrose, lactose and D-glucose that are heat resistant carbohydrates whereas, galactose, maltose, gelatin, cellulose and starch, those are of heat liable carbon sources and from sugar alcohol glycerol (Somasegaren and Hoben, 1994). One gram (1g) carbohydrate from each heat stable and heat liable was dissolved in 10ml of distilled water and was kept in refrigerator. Basal media (carbohydrate free media containing the following ingredients (g/l) was also prepared: K_2HPO_4 (1), KH_2PO_4 (1), Fe $Cl_3.6H_2O$ (0.01), $MgSO_4.7H_2O$ (0.2), $CaCl_2$ (0.1), $(NH_4)_2SO_4$ (1).

The heat stable carbohydrate sources were autoclaved together with the basal media. The heat liable carbon sources were first filter sterilized using sterile 0.22µm disposable membrane filters. They were then added to the autoclaved and cooled basal media. 48 hrs old rhizobial broth cultures were inoculated on to the basal media containing the different carbon sources (Somasegaren and Hoben, 1994).

Amino acid utilization

Different types of amino acids which include D-Alinine, Aniline, Urea and Arginine were used to determine the ability of the isolates to utilize amino acids. These amino acids were added at a concentration of 0.5 g / l to a similar basal media used for carbohydrate sources that lack ammonium sulfate and supplemented with 1g/ l of mannitol. The membrane filter sterilized amino acids were added to the autoclaved and cooled (approximately 55^{0}C) basal media as indicated in Amargar*et al.*, (1997). 48hrs old rhizobial suspensions were inoculated in to these basal media.

In vitro antifungal test

The chickpea pathogen, *Fusarium oxysporum* fs. ciceris was isolated from *fusarium* infected chickpea plant by growing them on PDA media and was identified morphologically using the standard identification keys of Barnett and Hunter (1998). The antagonistic activity of the rhizobia can be tested using dual culture technique as described by Landa *et al.*, (1997). Loopful of the bacterial isolates was equidistantly spot inoculated on the margins of potato dextrose agar (PDA) plates amended with sucrose (0.5%) and incubated at 28 ±2°C for 48 hrs. After 48 hrs, a 4mm *Fusarium oxysporum* fs. ciceris was placed at the center of the bacterial inoculated plate and incubated 28 ±2°C for 5- 7 days. The presence and absence of inhibition zone was recorded. Percent inhibition of radial growth of *F. oxysporum* f. sp. chickpea (PIRG) was calculated from the equation,

PIRG= C-T/Cx100 (Landa *et al.*, 1997),

Where, PIRG = percentage inhibition of radial growth, C = radial growth of *F. oxysporum* on control plate, T = radial growth of *F. oxysporum* on treatment plate.

Hydrogen Cyanide Production Test (HCN)

All isolates were tested for HCN production by streaking them on YEMA slant medium inserted with filter paper strips dipped in picric acid and 2% sodium carbonate according to Bakker and Schippers (1987). The test tubes were sealed with paraffilm. Finally, the slants were incubated for 5-7days at 28 ± 2 ^{0}C. Color change of the filter paper from deep yellow to orange and orange to brown was taken indicative of HCN production.

Production of ammonia test

Isolates were tested for the production of ammonia (NH_3) in peptone water. Freshly grown bacterial broth cultures were inoculated in 10 ml peptone water and be incubated in a rotator shaker at 28± 2°C for 3–5 days. Nessler's reagent (0.5 ml) was added to each tube. The formation of deep yellow to brown color develop was considered as positive test for ammonia production (Malleswari and Bagyanarayana, 2013).

Hydrolytic enzyme production test

Chitinase test

The potential of the rhizobial isolates to produce chitinase was determined by growing the isolates on chitin agar medium containing (g L-1): colloidal chitin (4), $MgSO_4.7H_2O$ (0.5), K_2HPO_4 (0.7), KH_2PO_4 (0.3), $FeSO_4.7H_2O$ (0.01), $MnCl_2$ (0.001), NaCl (0.3), yeast extract (0.2) and agar (20) using the method described by Nisa*et al.,* (2010). Loopful of the isolates were streaked and incubated at 28 ± 2°C for 48 h. The formation of clear Zone was recoded as chitinase producing.

Cellulase test

The ability of the rhizobial isolates to produce cellulase was determined by inoculating a loopful of 48 hrs grown rhizobial isolates on CMC agar medium containing g/l of ($NaNO_3$ (2), K_2HPO_4 (1), $MgSO_4$ (0.5), KCl (0.5), CMC sodium salt (2), Peptone (0.2), Agar (17), with 1000 ml distilled water (Kasana*et al.,* 2008). They were incubated at 28 ± 2^{0}C for 3-5 days. Clear zone surrounding the colonies were used as indicative of cellulase production.

Table.1 N free plant nutrient solutions

Stock solutions	Chemicals	g/liter
1	$CaCl_2.2H_2O$	294.1
2	KH_2Po_4	136.1
3	$FeC_6H_5O_7.3H_2O$	6.7
	$MgSO_4.7H_2O$	123.3
	K_2SO_4	87
	$MnSO_4.H_2O$	0.338
4	H_3BO_3	0.247
	$ZnSO_4.7H_2O$	0.288
	$CuSO_4.5H_2O$	0.1
	$CoSO_4.7H_2O$	0.056
	$Na_2MoO_2.2H_2O$	0.048

Source: Broughton and Dilworth, 1970

Protease test

The isolates potential to grow on YEMA media supplemented with skim milk (Skim milk powder 10g ml^{-1}, agar 15g ml^{-1}) was evaluated (Smibert and Krieg, 1994).They were incubated at 28±2^{0}C for 3-5 days. Protease activity was confirmed by the formation of clear zones around colonies.

Phosphate solubilizing test

Phosphate solubilizing ability was checked by inoculating each of them on Picovaskaya Agar medium containing (g/l): Glucose (10), Tricalcium phosphate (5), Ammonium Sulphate (0.5), Yeast extract (0.5), Magnesium Sulphate (0.1), Sodium Chloride (0.2),

Manganese Sulphate (0.002), and Agar (15). The pH of the medium was adjusted to 7.0. The medium was supplemented with 2.5 g/l of Ca_3 $(PO_4)_2$ (TCP) as P source. The growth and clear zone formation around the colonies were considered for phosphate solublization (Somasgaran and Hobben, 1994).

Invivo antagonistic Test of Few Selected Isolates under Greenhouse Condition

The soil was sieved, mixed and autoclaved and 3 kg is shared to each surface sterilized plastic pots. Four uniform surface sterilized seeds were planted to each pot and the treatments were laid down in complete randomized design. Bacterial inoculum was prepared in 250-ml flasks containing YEMB. After inoculation with bacteria, the flasks were incubated on a rotator shaker at 120 rpm and 28°C for 72 hr. After germination, the seedlings were inoculated with 48 hrs grown isolates. Inoculum of Foc race also obtained by transferring agar discs 4-mm in diameter cut from the edge of 7-day-old colonies growing on PDA, to 250ml flasks (one disk/flask) containing 100 g of 9:1 sand maize medium (Haware and Nene, 1982). After incubation for two weeks at 25°C, the flasks were used to inoculate a mixture of soil, sand and peat (1/1/1), sterilized by autoclaving twice at 110°C for 1.5 hrs in batches of 20 kg. The soil and contents of flasks (20:1 w/w) were mixed and the inoculated soil distributed to 15-cm plastic pots. After some weeks, the chickpea plants were infected with the chickpea pathogen, *Fusarium oxysporum* fs. ciceris which was isolated on PDA media. All plants were watered every two days. Finally, after 45 days cultivation, disease incidence was calculated using the formula as described by Brien (1991):

$$\text{Disease Incidence} = \frac{\text{number of infected plants}}{\text{Total number of plants}} \times 100$$

In conclusion the major goal of conducting a wide range of rhizobium research is to gain better strains for use in legume inoculants in agriculture with diverse agroecological zone. This pursuit entails the collection of isolates, strain characterization, assessment of symbiotic capacity and comparison to strains currently included within inoculants. From this review, it can be concluded that the selection of highly performed isolates using different consecutive steps of identification and authentication are worthy of further investigation of field try for rhizobilogical science. Symbiotic performance is a key but the ability of rhizobia to survive stress conditions or to utilize less expensive growth media are also important considerations. The process of rhizobial isolates exploration and characterization is somewhat arduous, and efforts must remain focused upon relatively few legumes of interest in an unbiased manner so that elite strains emerging from a work must be recognizably superior.

References

Bakker A.W., Schippers B. 1987. Microbial cyanide production in the rhizosphere in relation to potato yield reduction and Pseudomonas spp-mediated plant growth-stimulation. *J. of Soil Biology Biochemical*.19: 451-457.

Barnett, Horace Leslie, and Barry B. Hunter .1998.Illustrated genera of imperfect fungi. No. Ed. 4.American Phytopathological Society (APS Press).

Bauer, A.W., Kirby, W.M.M., Sherris, J.C. and Turck, M. 1966.Antibiotic susceptibility testing by a standardized single disk method. *American journal of clinical pathology*, 45:4, 493-496.

Gao, J.L., Sun, J.G., Li, Y., Wang, E.T. and Chen, W.X. 1994. Numerical taxonomy and DNA relatedness of tropical rhizobia isolated from Hainan Province, Chin. *Int. J. Syst. Bac.* 44:151-158.

Haware M.P., Nene Y.L. 1982. Races of *Fusarium oxysporum* f.sp.ciceris. *Plant Disease*66: 809-810.

Holt, J.G. 1994. Group 5, facultatively anaerobic gram-negative rods. *Bergey's manual of determinative bacteriology*.175-289.

Hungaria M, Andrade DS, and Chueira LM. 2000. Isolation and characterization of new efficient and competitive bean (*Phaseolus vulgaris* L.) rhizobia in Brazil. *Soil Biology and Biochemistry*.32: 1515-1528.

Jordan, D. C. (1984). *Bradyrhizobium*. *Bergey's manual of systematic bacteriology*. 1:242-244.

Kasana, R. C., Salwan, R., Dhar, H., Dutt, S. and Gulati, A. 2008. A Rapid and Easy Method for the Detection of Microbial Cellulases on Agar Plates Using Gram's Iodine. *Current Microbiol*.57:503–507.

Landa, B.B., Hervas, A., Bethiol, W. and Jimenez-Diaz, R.M. 1997.Antagonistic activity of bacteria from the chickpea rhizosphere against *Fusariumoxysporum* f. sp. ciceris.*Phytoparasitica*.25: 305–318.

Lupwayi, N. Z., and Haque, I. 1994.Legume-Rhizobium technology manual. *ILCA Environmental Sciences Working Document (ILCA)*. No. 29.

Malleswari, D. and Bagyanarayana, G. 2013. In vitro screening of rhizobacteria isolated from the

rhizosphere of medicinal and aromatic plants for multiple plant growth promoting activities. *J MicrobiolBiotechnol Res*. 3: 84–91.

Mhamdi, R., Jebara, M., Aouani, M.E., Ghrir, R. and Mars, M. 1999. Genotypic diversity and symbiotic effectiveness of rhizobia isolated from root nodules of *Phaseolus vulgaris* L. grown in Tunisian soils. *Biol. and fertility of soils*.28:3, 313-320.

MussaAdaland FasilAsefa.2010. Phenotypic and Symbiotic characterization of grass pea (Lathyrussativus) rhizobial isolates from some major growing areas of South Wollo and West Shoa , Ethiopia. MSc. thesis. Addis Ababa University, Addis Ababa, unpuplished, Ethiopia.

MussaAdal. 2018. Diversity, Symbiotic and Plant Growth Promoting Properties of Rhizobia and Rhizobacteria of Grass Pea *(LathyrusSativus L.)* From Central Ethiopia: Implication to the Selection and Use of Microbial Inoculants in Low Input Agriculture in Ethiopia (Doctoral dissertation, Addis Ababa University).

Nurdebyandaru, Nisa, Mubarik, N.R. and Prawasti, T.S. 2010. Chitinolytic bacteria isolated from Chili rhizosphere: chitinase characterization and application as biocontrol for *Aphis gossypii*. *Microbiol. Indonesia*, 4:3, 18-2.

Ocamb, C.M., Buschena, C.A. and O'Brien, J. 1997.Microbial mixtures for biological control of *Fusarium* diseases of tree seedlings. *United States Department Of Agriculture forest service general technical report pnw*. 159-166.

Smibert, R.M., Krieg, N.R., Gerhardt, P., Murray, R. and Wood, W.A. 1994. Methods for general and molecular bacteriology. American Society for Microbiology, Washington DC.

Somasegaran, P., and Hoben, H. J. 2012. *Handbook for rhizobia: methods in legume-Rhizobium technology*. Springer Science and Business Media.source of nitrogen for sustainable agricultural production.*Plant Soil.*174:3-28.

Somasegaren, P. and Hoben, H. J. 1994. Hand book for rhizobia. Methods in legume-*Rhizobium* technology. Springer verlag, New York. 1-441.

Vincent, J. M. 2001. A manual for the practical study of the root-nodule bacteria. *A manual for the practical study of the root-nodule bacteria.*

Vincent, J.M. 1970. A manual for the practical study of the root-nodule bacteria. *A manual for the practical study of the root-nodule bacteria.*

Vinuesa, P., Silva, C., Werner, D. and Martinez-Romero, E. 2005a. Population genetics and phylogenetic inference in bacterial molecular systematics: the roles of migration and recombination in *Bradyrhizobium* species cohesion and delineation. *Mol. Phyl. Evol*.34: 29-54.

White, D. 1995. The physiology and Biochemistry of prokaryotes. Oxford University Press. 34-46.

Broughton, W.J. and Dilworth, M.J. 1971.Control of leg-haemoglobin synthesis in snake beans. *Biochemical journal.* 125:4, 1075-1080.

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