www.ThePharmaJournal.com

The Pharma Innovation

ISSN (E): 2277- 7695
ISSN (P): 2349-8242
NAAS Rating 2017: 5.03
TPI 2017; 6(9): 368-371

www.thepharmajournal.com
Received: 05-07-2017
Accepted: 07-08-2017

Rakesh Patel
Department of Life Science, Shree Rewa Gurjar Ball Niketan, College, Khargone Road Sanawad, Madhya Pradesh, India

Gopal Rathor
Department of Life Science, Shree Rewa Gurjar Ball Niketan, College, Khargone Road Sanawad, Madhya Pradesh, India

Bhupendra H Bhargav
Department of Life Science, Shree Rewa Gurjar Ball Niketan, College, Khargone Road Sanawad, Madhya Pradesh, India.

K Singh
Department of Life Science, Shree Rewa Gurjar Ball Niketan, College, Khargone Road Sanawad, Madhya Pradesh, India.

Davendra bharti
Department of Life Science, Shree Rewa Gurjar Ball Niketan, College, Khargone Road Sanawad, Madhya Pradesh, India.

Correspondence
Rakesh Patel
Department of Life Science, Shree Rewa Gurjar Ball Niketan, College, Khargone Road Sanawad, Madhya Pradesh, India.

Study and effect of *Rhizobium* bacteria and culture suspension isolated from root nodules at Nimad Area

Rakesh Patel, Gopal Rathor, Bhupendra H Bhargav, K Singh and Davendra Bharti

Abstract
Rhizobium species are group of bacteria that fix atmospheric nitrogen symbiotically and stimulate the growth of plants. In the present study, root nodulating bacteria were isolated from root nodules of *Cicer arietinum* on yeast extract mannitol agar (YEMA) medium. In conical flask assays of *Rhizobium*, seed inoculation was more effective than soil inoculation. The effect of inoculation of *Rhizobium* on nodulation and growth of *Cicer arietinum* was also investigated using a water agar medium under laboratory conditions. In case of seed germination on the growth of Bengal gram (*Cicer arietinum*), crop plants, it was noted that no enhanced growth rate was obtained; hence *Rhizobium* may be used as cobioinoculant with other banificial bacteria and micronutrient. Use of micro and macro nutrient may improve effectiveness of *Rhizobium* biofertilizers for common bean production. Testing of *Rhizobium* under field conditions will further elucidate their effectiveness on grain yields of *Cicer arietinum* in Nimar area with different physical condition and nutrient.

Keywords: rhizobacteia, PGPR, Culture, Becteria, YEMA

Introduction
Rhizobium is a group of bacteria that fix atmospheric nitrogen symbiotically and stimulate & support the growth of plants. Chahboune *et al.*, 2011 [2] observed that native bacterial population, is very important to understand the characterization, and identification of distribution and diversity of indigenous bacteria in the rhizosphere of specific crops. Deepa *et al.*, 2010 [3] noted region-specific microbial strains that were used as growth promoting/enhancing inoculum to achieve desired crop production and their uses replace the use of chemical bio fertiliser. Ahmad *et al.* 2009 [1] noticed that the bacterial diversity in the forest soil of Kashmir, India was investigated but no data is available regarding the rhizosphere microbiome of gram crops in Nimad area. Similarly, legume growth and yields have been shown to increase with inoculation with Rhizobia. Co-inoculation with P-solubilizing bacteria and *Rhizobium* stimulated plant growth more than their separate inoculations (Morel *et al.*, 2012; Walpola and Yoon, 2013) [15]. Hezekiah Korir *et al.* 2017 [11] confirmed the difference between the co-inoculation of rhizobia strains and PGPR compared to single rhizobia inoculation on the root dry weight. These results show that coinoculation of PGPR and Rhizobia has a synergistic effect on bean growth. Use of PGPR may improve effectiveness of *Rhizobium* biofertilizers for common bean production. Hayat *et al.*, 2010 [10] were seen that the bacteria lodging around the plant roots (rhizobacteria) are more versatile in transforming, mobilizing, solubilizing the nutrients compared to those from bulk soils.
Toniutti *et al.* 2017 [22] found that local isolates were more adjustable with high temperatures, extreme pH and salinity in Argentinean soils and conclude that local isolates could be efficiently used for as inoculation. Moyano *et al.* 2017 [16] shown that the cheating partners (bacteria) increasing experimental evidence and show mutualisms in with other life form. Harish kumar *et al.* 2011 [9] selected Five bacterial strains (TR1 to TR5) from root nodules of fenugreek (Trigonella foenum-graecum) for the work on plant growth promotory traits and biocontrol potential against Fusarium oxysporum and finally the conclude rhizobial isolates have properties of biocontrol agents and may be applied to promote the growth Fusarium wilt with fenugreek. Hussain *et al*., 2009 [12] observed the importance of *Rhizobium* increases plant growth by various ways such as production of plant growth hormones, vitamins, siderophores, by solubilisation of insoluble phosphates, induction of systemic disease resistance and enhancement in stress resistance.

This availability of this bacteria interaction reduces the need of nitrogenous fertilizers during the growth of leguminous crops, the were help in balancing various pollution and ecological factors. Olivera *et al.* 2011 [17] have seen that Improvement of common bean growth by co-inoculation with *Rhizobium* and plant growth-promoting bacteria and also ability to improve nutrient uptake efficiently. Junkang *et al.* 2014 [13] observed the levels of Cd-tolerant PGPR can alleviate Cd toxicity to the plants, increase Cd accumulation in *L. multiflorum* Lam. by enhancing Cd availability in soils and plant biomass, but decrease Cd accumulation in *G. max* (L.) Merr. Enhanced plant growth, nodulation and nitrogen fixing parameters were observed in chickpea with inoculation of endophytic isolates in combination with *MesoRhizobium* (Saini *et al.* 2005). Chickpea (*Cicer arietinum*) is the world`s third most important food legume with high in protein and one of the earliest cultivated legumes. But still chickpea and its nodule bacteria need to be studied more carefully. Endophytic and rhizospheric Bacteria were studied and observed the play important role in plant yield and growth promotion, plant health, and protection (Hallmann and Berg, 2006; Ryan *et al.*, 2008; Dudeja and Giri, 2014; Saini *et al.*, 2015; Dudeja, 2015) [8, 20, 5 19, 4]. Author used of microorganisms with the ability to solubilize mineral and organic P (Khan *et al*., 2006; Fernández *et al*., 2007 Shiri-Janagard *et al*., 2012) [14, 7, 21] or to fix nitrogen (Uribe *et al*., 2012) [23].

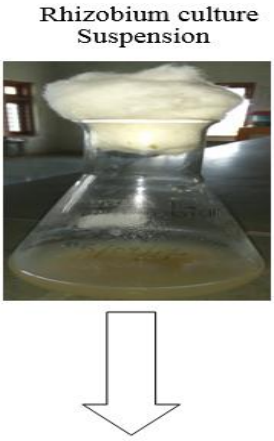
In the present study we considered rhizobacteria isolated from root nodules and their culture suspension applied on water agar growing gram plant and their response was compared with control group. In India, specially in Khargone district of Madhya Pradesh much emphasis in bio fertilization of legumes has been put on rhizobia inoculation, particularly for common bean. Importantly, in Khargone district, information is scanty regarding isolation of native root endogenous rhizobia and the role in phosphorus bioavailability, growth promotion and also their interaction with rhizobia in common bean. The present study was therefore designed to isolate and identify *Rhizobium* from common bean root nodules and evaluate the effect of inoculation of Rhizobia on growth and nodulation of common beans in a water agar media in laboratory. Finally, the study help to understand latest beneficial rhizobacteria in different agro-ecosystems have been presented comprehensively under both normal and stress micronutrient to highlight the recent trends with the aim to develop future insights.

Materials and Methods

Isolation and Identification of Rhizobium

The experiment was conducted using root nodules from one locations (Khargone M.P.) in India to chickpea or chick pea (*Cicer arietinum).* Root nodules were obtained from fields in which chickpea or chick pea (*Cicer arietinum)* had recently been cultivated. Fifteen root nodulating bacteria were isolated from nodules of chickpea or chick pea (*Cicer arietinum)* according to the previously develop method and were subjected to growth and were maintained on yeast extract mannitol agar (YEMA). Purity of colonies was checked for by repeated streaking on YEM plates and by microscopic examination of living cells. Microscopic observations were performed to investigate some characteristics of the isolates such as shape and gram reaction. Catalase test was also carried out using 24 h old bacterial cultures whereby a single bacterial colony was placed on glass slide and a drop of 30% hydrogen peroxide (H_2O_2) added. Appearance of gas bubbles indicated the presence of catalase enzymes in the bacteria. A pure culture was grown in YEM. Water agar medium were used for the growth and support for seed germination and plant growth. Seeds were surface-sterilized by soaking in 3.5% NaOCl solution for 5 minutes and then thoroughly washed with sterile, distilled water. Two pre-selected healthy seeds of uniform size were then planted per conical flask, and thinned to one plant per conical flask of comparable germination and height between 1 and 2 days after planting. Plants flask were observed daily and twice daily (if necessary) during later growth stages to measured height in flask. There were no standard nutrient solution containing macronutrients K, Mg, Ca, and S, and micronutrients Mn, Zn, Cu, B, Mo, considered for study. Flasks were grown under normal conditions at room temperature.

Fig 1: *Rhizobium* colonies observed on YEM.



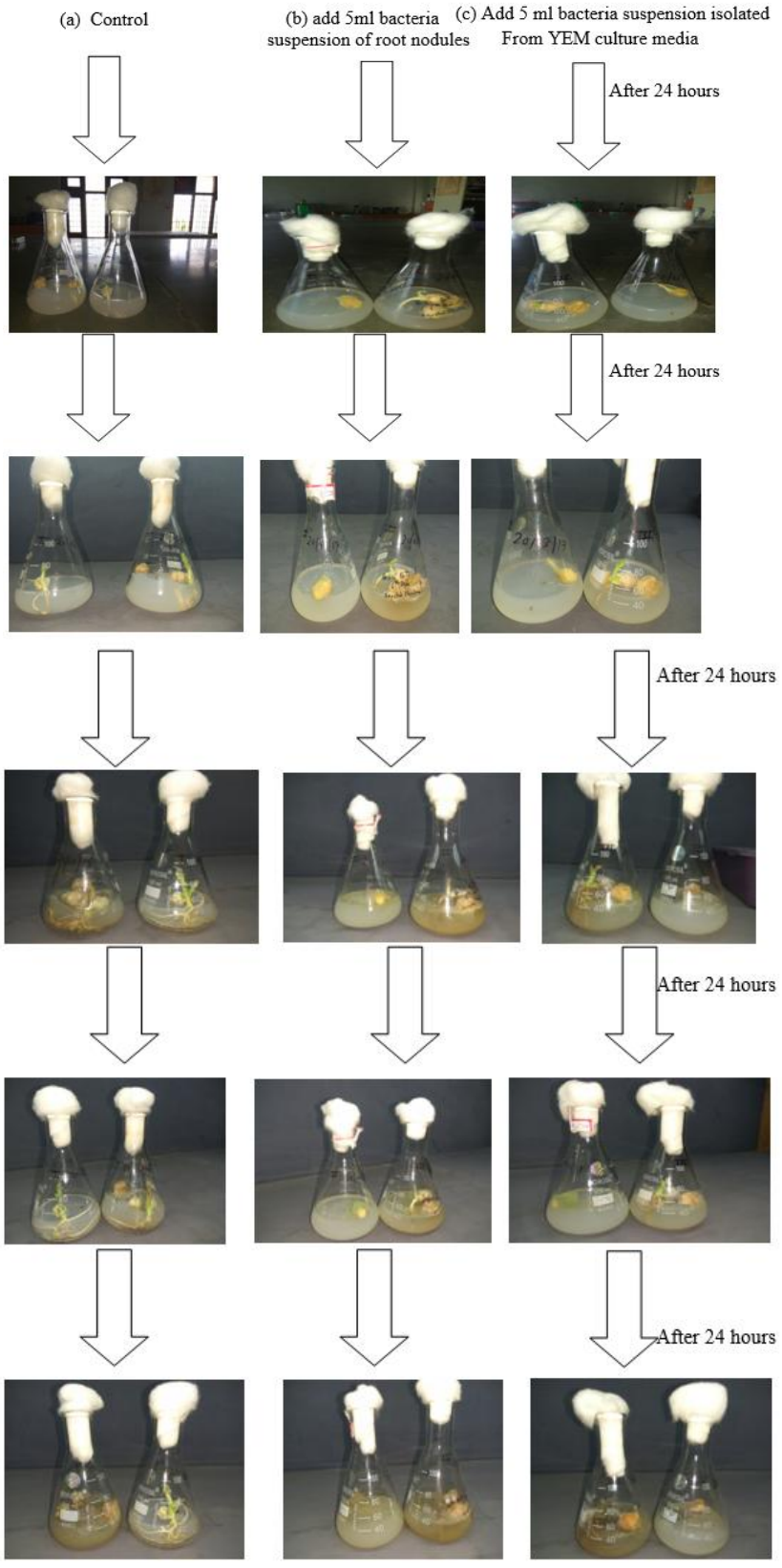


Fig.2 Growth promotion of chickpea (*Cicer arietinum)* after inoculation with root nodules isolated *Rhizobium*. (a) Control, (b) bacteria suspension from root nodules (c) Bacterial suspension from YEM culture.

Result and Discussion

Our comparative research and understanding of mechanisms of rhizobia mediated-phyto stimulation way to find out more competent rhizobacterial strains which may work under diverse agro-ecological conditions. Isolated bacterium was proved as *Rhizobium* based on their cultural, morphological and biochemical characteristics. These results summarized that *Rhizobium* can be effectively used as a bioinoculant and micro & macro nutrient to enhance the yield of crops. We add the culture suspension of *Rhizobium* after germination the seed and no visible effect were seen on plant growth and may suggested that bacteria need proper micronutrient environment and pretreatment of seed with biofertilizer may suggest proper growth.

Acknowledgement
Authors sincerely acknowledge Shree Rewa Gurjar Bal Niketan college Sanawad for financial assistance and facility.

References

1. Ahmad N, Johri S, Abdin MZ, Qazi GN. Molecular characterization of bacterial population in the forest soil of Kashmir, India. World J Microbiol. Biotechnol. 2009; 25:107-113.
2. Chahboune R, Barrijal S, Moreno S, Bedmar EJ. Characterization of *BradyRhizobium* species isolated from root nodules of *Cytisus villosus* grown in Morocco. Syst. Appl. Microbiol. 2011; 34,440-445.
3. Deepa CK, Dastager SG, Pandey A. Isolation and characterization of plant growth promoting bacteria from non-rhizospheric soil and their effect on cowpea (*Vigna unguiculata* (L.) Walp.) seedling growth. World J Microbiol. Biotechnol. 2010; 26:1233-1240.
4. Dudeja SS. Beneficial effects and molecular diversity of endophytic bacteria in legume and non-legumes In: Singh DP, Abhilash PC, Ratna Prabha. (Eds), Microbial inoculants in sustainable agricultural productivity, Research perspectives. Springer-Verlag, Germany (In press), 2015, I.
5. Dudeja SS, Giri R. Beneficial properties, colonization, establishment and molecular diversity of endophytic bacteria (review). Afr. J Microbiol. Res. 2014; 8(15):1562 1572.
6. Ei-Yazeid AA, Abou-Aly HE. Enhancing growth, productivity and quality of tomato plants using phosphate solubilizing microorganisms. Aust. J Basic Appl. Sci. 2011; 7:371-379.
7. Fernández LA, Zalba P, Gómez MA, Sagardoy MA. Phosphatesolubilization activity of bacterial strains in soil and their effect on soybean growth under greenhouse conditions. Biol. Fertil. Soils. 2007; 43:805-809. doi: 10. 1007/s00374-007-0172-3.
8. Hallmann J, Quadt-Hallmann A, Mahaffee WF, Kloepper JW. Bacterial endophytes in agricultural crops. Can. J Microbiol. 1997; 43:895-914.
9. Harish Kumar, Dubey RC, Maheshwari DK. Effect of plant growth promoting rhizobia on seed germination, growth promotion and suppression of Fusarium wilt of fenugreek (*Trigonella foenum-graecum* L.) Crop Protection. 2011; 30:1396-1403.
10. Hayat R, Ali S, Amara U, Khalid R, Ahmed I. Soil beneficial bacteria and their role in plant growth promotion: a review. Ann Microbiol. 2010; 60:579-598.
11. Hezekiah Korir, Nancy W Mungai, Moses Thuita, Yosef Hamba, Cargele Masso. Co-inoculation Effect of Rhizobia and Plant Growth Promoting Rhizobacteria on Common Bean Growth in a Low Phosphorus Soil. 2017; 8:141.
12. Hussain MB, Mehboob I, Zahir ZA, Naveed M, Asghar HN. Potential of *Rhizobium* spp. for improving growth and yield of rice (*Oryza sativa* L.). Soil Environ. 2009; 28(1):49-55.
13. Junkang Guo, Jie Chi. Effect of Cd-tolerant plant growth-promoting *Rhizobium* on plant growth and Cd uptake by *Lolium multiflorum* Lam. and *Glycine max* (L.) Merr. in Cd-contaminated soil. Plant and Soil. 2014; 375:205-214
14. Khan MS, Zaidi A, Wani PA. Role of phosphate solubilizing microorganisms in sustainable agriculture-A review. Agron. Sustain. Dev. 2006; 26:1-15.
15. Morel MA, Braña V, Castro-Sowinski S. Legume crops, importance and use of bacterial inoculation to increase production. Crop Plant. 2012; 12:218-240.
16. Moyano G, Marco D, Knopoff D, Torres G, Turner C. Explaining coexistence of nitrogen fixing and non-fixing rhizobia in legume-rhizobia mutualism using mathematical modeling. Math Biosci. 2017; 292:30-35.
17. Olivera Stajkovic, Dusica Delic, Dragana Josic, Đorde Kuzmanovic, Natasa Rasulic, Jelena Knezevic-Vukcevic. Improvement of common bean growth by co-inoculation with *Rhizobium* and plant growth-promoting bacteria Received for publication, 2011, 16.
18. Phosphate solubilizing bacteria: assessment of their effect on growth promotion and phosphorous uptake of mung bean (Vigna radiata [L.] R. Wilczek). Chil. J Agric. Res. 2012; 73:275-281.
19. Ranjana Saini, Vishal Kumar, Dudeja SS, Pathak DV. Beneficial Effects of Inoculation of Endophytic Bacterial Isolates from Roots and Nodules in Chickpea. 2015; 4:207-221.
20. Ryan RP, Germaine K, Franks A, Ryan DJ. Bacterial endophytes: recent developments and applications. FEMS Microbiol. Lett. 2008; 278:1- 9.
21. Shiri-Janagard M, Raei Y, Gasemi-Golezani G, Aliasgarzad N. Influence of Brady*Rhizobium* japonicum and phosphate solubilizing bacteria on soybean yield at different levels of nitrogen and phosphorus. Int. J Agron. Plant Prod. 2012; 3:544-549.
22. Toniutti MA, Fornasero LV, Albicoro FJ, Martini MC, Draghi W, Alvarez F *et al*. Nitrogen-fixing rhizobial strains isolated from Desmodium incanum DC in Argentina: Phylogeny, biodiversity and symbiotic ability. Syst Appl Microbiol. 2017; 40(5):297-307.
23. Uribe D, Sanchez-Nieves J, Vanegas J. Role of microbial biofertilizers in the development of a sustainable agriculture in the tropics, in Soil Biology and Agriculture in the Tropics, ed. P. Dion (Berlin: Springer), 2012, 240-250.