Available online at www.ijpab.com

DOI: http://dx.doi.org/10.18782/2320-7051.6058

ISSN: 2320 – 7051

Int. J. Pure App. Biosci. **6 (2):** 142-145 (2018)

Research Article

International Journal of Pure & Applied Bioscience

OPEN ACCESS

Siderophore Production and Biocontrol Potential of Rhizobium Isolated from Non- Traditional Leguminous Crop in M.P.

Radha Gupta[1*], Shashi S. Yadav[2], S. K. Verma[2] and S. K. Dubey[2]

[1]Department of Plant molecular Biology and Biotechnology, [2]Department of Soli Science, College of Agriculture, RVRSKVV, Gwalior, M.P. India

*Corresponding Author E-mail: radhagi80@rediffmail.com

Received: 1.12.2017 | Revised: 6.01.2018 | Accepted: 10.01.2018

ABSTRACT

An attempt has been made to evaluate siderophore and biocontrol activities in Rhizobia were isolated from non-traditional leguminous crops cowpea, moth and guar from various regions of Madhya Pradesh. Siderophore activity was achieved by chrome azurol sulfonate (CAS) assay, a universal siderophore detection method. Formation of orange halos in blue agar plates confirmed the CAS assay and comparison of halos diameter of Rhizobial strain revealed that only two strains R-3 and R-8 were effective in siderophore production from eight Rhizobial strain and only one isolate R-6 showed biocontrol activity against two fungal strains R. solanii and S. sclerotiorum.

Key words: *Biocontrol activity, Rhizobium and Siderophore.*

INTRODUCTION

Soil contains many types of microorganisms such as bacteria, actinomycetes, fungi and algae which are important because they affect the physical, chemical and biological properties of soil[2]. Amongst the soil bacteria *Rhizobia* has a beneficial effect on the growth of plants abd live either in the soil or within the root nodules of host legumes. Symbiotic relationship undergoes in leguminous plant and the relationship is iron dependent, nodule formation require iron as well as nitrogenase system and leghaemoglobin for nitrogen fixation[11]. Siderophores are relatively low molecular weight, ferric ion specific chelating agents produced by bacteria growing under low iron stress. These compounds scavenge iron from the environment and make the mineral available to the microbial cell[4]. Under aerobic conditions microorganisms needs iron for a variety of functions including reduction of oxygen for synthesis of ATP, reduction of ribonucleotide precursors of DNA, for formation of heme and other essential purposes. But aerobic atmosphere caused surface iron to oxidiz to insoluble oxyhydroxide Polymer and reduced the level of free iron, hence bacteria choose the way for iron uptake by producing iron chelating molecule known as siderophore[7]. The compound is secreted by bacteria solublize and bind iron and transport back into microbial cell. *Rhizobium* is an antagonist bacteria and also having biocontrol potential[3].

Cite this article: Gupta, R., Yadav, S.S., Verma, S.K. and Dubey, S. K., Siderophore Production and Biocontrol Potential of Rhizobium Isolated from Non- Traditional Leguminous Crop in M.P., *Int. J. Pure App. Biosci.* **6(2):** 142-145 (2017). doi: http://dx.doi.org/10.18782/2320-7051.6058

Present study aims to evaluate siderophore and biocontrol potential of *Rhizoba* from non traditional leguminous crops in Madhya Pradesh (M.P.).

MATERIAL AND METHODS

Collection of Rhizospheric soil and plant samples:

Rhizospheric soil and various non traditional leguminous plants cowpea, moth and guar were collected from Gwalior, Morena, Shivpuri, Bhitarwar, Guna, Ujjain, Jhabua, Jobat and Alirajpur districts of M.P.[6]

Root nodule sampling and Rhizobial isolation:

Fresh, healthy root nodules and soil sample were collected and isolate from field of three species of non-traditional legumes (cowpea, guar and moth) according to Dubey and Gupta[6].

Siderophore Production:

Test tubes (Borosil, 18X150 mm) containing 5ml YMB were inoculated with Rhizobial isolates. The tubes were then incubated at 28°C in incubator shaker (New Brunswick Scientific, Edison, NJ, USA, Innova Model 4230 refrigerated incubator shaker) at 180 rpm for 48 hours. 10 µl of the suspension was spotted in wells on CAS Agar media plates and incubated at 28^0C in BOD incubator (Composite Lab line, India) for 48 hours. The plates were then observed for orange halo around the colonies[13].

HCN Production:

24 hrs old cultures were stabbed on YMA plates supplemented with 0.45% glycine. A sterilized filter paper saturated with 1% picric acid and 2% sodium carbonate was placed in the upper lid of Petridish. The plates were sealed with parafilm and incubated at 30°C for 4 days. The filter paper was observed for any change in color from yellow to reddish brown as an indication of cyanogenic activity.

RESULTS AND DISCUSSION

A number of isolates were obtained from root nodules of cowpea (four isolates R-1, R-2, R-3 and R-4), moth (two isolates R-5 and R-6) and guar (two isolates R-7 and R-8) from Gwalior, Shivpuri, Guna, Ujjan, Jhabua, Alirajpur and Dhar districts of M.P. These isolates were primary characterized for their morphological characteristics and grouped on the basis of their common phenotypic and growth parameters. Formation of orange halos in blue agar plates confirmed the CAS assay and comparison of halos diameter of *Rhizobial* strain, revealed that few strain were effective in siderophore production (Fig. 1).The results indicated (Table 1) that only two strains R-3 and R-8 of *Rhizobia* showed positive siderophore activity and none of the isolates produced hydrocyanic acid. Similar result was reported by Carson *et al*[4]., in root nodule forming bacteria. Earlier studies have also reported a very low incidence of cyanogens in *Rhizobia* and in other PGPR[1]. It has also been reported that production of HCN proved to be deleterious to the plant.Biocontrol activity of all Rhizobial strain also reported against various fungal strain *F. oxysrum, F. qubens, R. solanii and S. sclerotiorum.* In eight *Rhizobia* isolates only one isolate R-6 show biocontrol activity against two fungal pathogen *R. solanii and S. sclerotiorum* (Table-1). Hameeda *et al.*[8] is also reported biological control of chickpea collar rot by co-inoculation of antagonistic bacteria *Rhizobia*. Siderophore production is one of the important traits of PGPR and is driving much attention since last few decades due to applications of siderophores in various other fields apart from agriculture. These isolates were screened for their siderophore and biocontrol activity and will be used for the further study.

Table 1: Siderophore, HCN production and Biocontrol potential of Rhizobial strains

Strain No.	Siderophore production	HCN production	Fungal Pathogens			
R-1	-	-	***F. oxysrum***	***F. qubens***	***R. solanii***	***S. sclerotiorum***
R-2	-	-	-	-	-	-
R-3	+	-	-	-	-	-
R-4	-	-	-	-	-	-
R-5	-	-	-	-	-	-
R-6	-	-	-	-	-	-
R-7	-	-	-	-	+(5 mm)	+ (8 mm)
R-8	+	-	-	-	-	-
			-	-	-	-

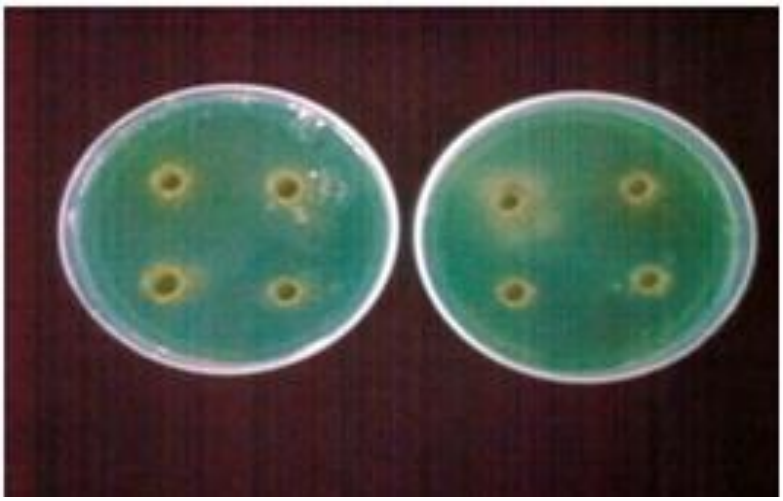

Fig. 1: Siderophore Production in Rhizobial strain R-3 and R-8

Acknowledgement

We would like to thank MPCOST, Bhopal, for providing financial support and and College of Agriculture, RVSKVV, Gwalior, India for support.

REFERENCES

1. Antoun, H., Beauchamp, C. J., Goussard, N., Chabot, R. and Lalande, R., Potential of *Rhizobium* and *Bradyrhizobium* species plant growth promoting Rhizobacteria on host legumes effect on radishs (*Raphanussativus L*). *Plant Soil* **204:** 57–67(1998).
2. Atlas, R. and Bartha, R., Microbial Ecology: Fundamentals and Applications. pp 694.4th Ed.Benjamin Cummings. Menlo Park, Canada (1998).
3. Bloemberg, G. V. and Lugtenberg B. J. J., Molecular basis of plant growth promotion and biocontrol by Rhizobacteria, *Current Opinion of Plant Biology* **4:** 343 – 350 (2011).
4. Carson, K. C., Holliday, S., Glenn, A.R. and Dilworth, M. J., Siderophore and organic acid production in root nodule bacteria. *Archives of Microbiology* **157:** 264-27 919920.
5. Cornelis, P. and Matthijs, S., Diversity of siderophore-mediated iron uptake systems in fluorescent *Pseudomonads*: not only pyoverdines. *Environmental Microbiology* **4:** 787-798 (2002).
6. Dubey, S. K. and Gupta, R., Characterization of Rhizobium from various non-traditional arid legume crops in M.P. *Journal of food Legumes* **28(1):** 90-93 (2015).
7. Ghorpade, V.M. and Gupta, S.G., Siderophore Production by Rhizobium nepotum isolated from "Stem nodule of

Aeschynomene indica, *Int. J. Adv. Res. Biol. Sci.* **3(7):** 105-108 (2016).

8. Hameeda, B., Harini, G., Rupela, O. P., Kumar, Rao, J. V. D. K. and Reddy G., Biological control of Chickpea collar rot by co-inoculation of antagonistic bacteria and compatible Rhizobia. *Indian Journal of Microbiology* **50 (4):** 419–424 (2010).
9. Louden, B. C., Haarmann, D. and Lynne, A. M., Use of Blue Agar CAS Assay for Siderophore Detection. *Journal of Microbiology and Biology Education* **12(1):** 51-53 (2011).
10. Mensah, J. K., Esumeh, F., Iyamu, M. O. C., Effects of different salt concentrations and pH on growth of *Rhizobium* sp. and a cowpea *Rhizobium* association. American-*Eurasian Journal of Agriculture Environmental Sciences* **3:** 198-202 (2006).
11. Raychaudhuri, N., Das, S. K. and Chakraborty, P. K., Symbionic Effectiveness If Siderophore Overproducing Mutant of Mesorhizobium ciceri. *Polish J Microbial*, **54:** 37-4 (2005).
12. Sayyed, R. Z., Badgujar, M. D., Sonawane, H. M., Mhaske, M. M. and Chinchokar, S. B., Production of Microbial iron chelators (siderophores) by fluorescent Pseudomonads. *Indian Journal of Biotechnology* **4:** 484-490 (2005).
13. Schwyn, B. and Neilands, J. B., Universal chemical assay for the detection and determination of siderphore. *Analytical Biochemistry* **160:** 47–56 (1987).