Plant Archives Volume 20 No. 1, 2020 pp. 2605-2608 **e-ISSN:2581-6063 (online), ISSN:0972-5210**

SIDEROPHORE PRODUCTION BY INDIGENOUS BACILLUS ISOLATE AND ITS ROLE IN GROWTH OF *CICER ARIETINUM*

G. Sai Krishna, S. Shalini Devi and K. Anuradha*

Dept of Microbiology, Bhavan's Vivekananda College of Science, Humanities & Commerce, Sainikpuri, Secunderabad-500 094 (Telangana), India.

Abstract

Siderophore producing bacteria are believed as ecofriendly alternatives for chemical fertilizers. The present study was aimed at isolating native bacterial isolates with plant growth promoting traits. We screened 4 bacterial isolates *viz.*, *Pseudomonas* RZ-2, *Pseudomonas* RZ-1 and *Pseudomonas* RZ-3 and *Bacillus* DY. All these four bacterial isolates were found to produce siderophores and IAA. Pot culture experiment performed by using *Chick pea* as model plant, grown in soil inoculated with PGP *Bacillus* DY isolate significantly enhanced the plant growth by increasing plant root length, plant shoot length, over the uninoculated control.

Key words: Siderophore, PGPR, Chick pea and IAA

Introduction

Iron is regarded as an essential nutrient for plant growth as it plays a vital role in synthesis and functioning of photosynthetic apparatus (Broadley *et al.*, 2012). Deficiency of this element can lead to plant chlorosis, characterized by yellowing of leaves (Lucena, 2000). Chlorosis can lead to severe yield losses or complete crop failure. Recent strategies to manage iron deficiency in plants included discovery of dominant chlorosis phenotypes (Harrington *et al*., 2019) and foliar application of iron fertilizers (Ahmad *et al*., 2016) and use of aminopolycarboxylic acids (APCAs) (Bucheli-Witschel and Egli , 2001) however deploying microbes for chelation of iron present in the soil could be a sustainable way for proper utilization of iron.

Although many research studies reported the isolation of siderophore producers, as the survival and multiplication of microbes is dependent on chemical, physical characteristics of soil and climatic conditions of a particular geographical area there is a need to screen native siderophore producers. The present research work was aimed at isolating iron cheating microbes from various regions of Hyderabad, Telangana, India.

Materials and Methods

Collection of soil samples and screening of siderophore producing bacteria.

Soil samples were collected from dump yards and agricultural fields present in Hyderabad, Telangana, India. Soil samples were serially diluted, plated on nutrient agar medium and incubated for 24 hrs at 37°. Morphologically varied bacterial colonies were subcultured on nutrient agar slants and stored for further use. Each of these bacterial colonies were spot inoculated on chrome azurol S (CAS) agar medium and incubated for 72 hrs at 37°C (Raaska *et al*., 1993).

Detection of siderophores in liquid cultures

Bacterial cultures showing zone of chelation were inoculated into nutrient broth and incubated at 37°C for 24hrs, culture broth was centrifuged for 10 min at 10000 rpm. To 1ml of culture supernatant , 100µl of CAS reagent (CAS 6.5 mg) dissolved in 5 ml of distilled water and mixed with 1ml of iron (III) solution (0.003g $FeCl.6H_2O$ in 0.9ml HCl of 10 ml distilled water). This solution is slowly added to 4ml of hexadecyltrimethylammonium bromide (HDTMA) (7.2 mg) was added) was added. Discolouration of blue/green coloured CAS reagent indicates presence of siderophores (Schwyn and Neilands, 1987).

Detection of IAA Production

The isolates were inoculated into nutrient broth (NB) containing 0.1% DL-tryptophan and incubated at 37°C

***Author for correspondence :** E-mail : anuradhapmv@gmail.com

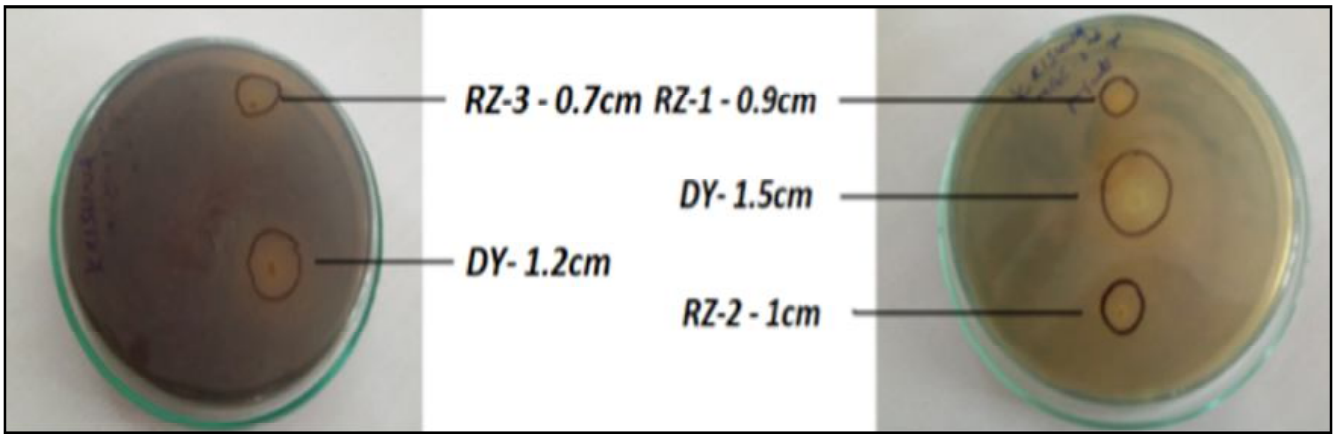

Fig. 1: Zone of chelations observed by the selected bacterial isolates with CAS assay.

Table 1: Table showing Siderophore and Indole Acetic Acid production at different time intervals by selected isolates.

TIME	*RZ-1*		*RZ-2*		*RZ-3*		*DY*	
Characteristic	**SP**	**IAA**	**SP**	**IAA**	**SP**	**IAA**	**SP**	**IAA**
4th hour	-	-	-	-	-	-	-	-
8th hour	-	-	-	-	-	-	-	-
12th hour	-	-	-	-	-	-	-	-
16th hour	+	-	+	-	-	-	+	+
24th hour	+	+	+	+	+	+	+	+

SP- Siderophore Production; IAA- Indole Acetic Acid.

Table 2: Root and Shoot length of chick pea plants grown in inoculated and uninoculated soils.

	Inoculated with Bacillus DY	**Uninoculated Control**
Average length of root	16cm	9.1cm
Average length of shoot	31.9cm	28.6cm

for 24hrs. The culture broth was centrifuged at 10,000rpm for 10 min at 4°C. The supernatant (2ml) is mixed with two drops of ortho phosphoric acid and 4ml of the salkowaski reagent (50 ml of 35% percholoric acid, 1ml 0.5M $FeCl_3$ solution) and incubated in dark at room temperature for 30-40 minutes. Development of pink color indicates IAA production (Gordon and Weber, 1951).

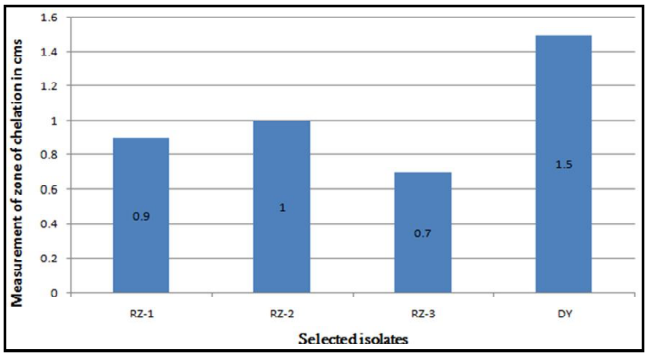

Fig. 2: Zone of chelation in cms produced by bacterial isolates.

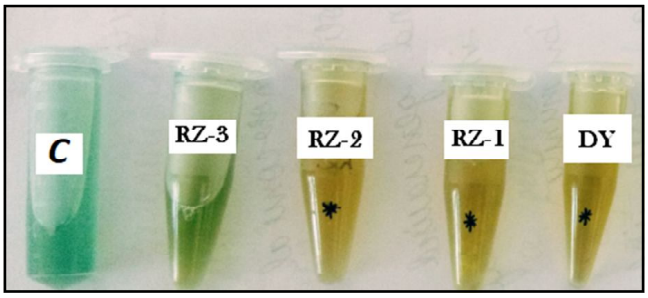

Fig. 3: Photograph showing discolouration of CAS reagent by siderophore producing bacterial isolates.

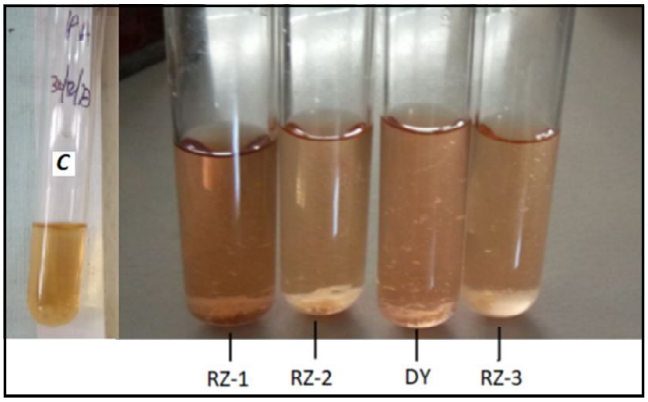

Fig. 4: IAA production of selected bacterial isolates.

Effect of incubation time on Siderophore and IAA production

Selected isolates were inoculated in nutrient broth and incubated for 24hrs at 37°C. The effect of incubation time on siderophore and IAA production was studied by qualitative detection of Siderophore and IAA production at 4th, 8th, 16th and 24th hrs of incubation.

Plant growth promotion potential of bacterial isolate, *Bacillus* DY.

The ability of *Bacillus* DY isolate in promoting plant growth was investigated using *Cicer arietinum* (Chickpea) as a model plant by following the method of Shalini *et al.*, (2019).

Fig. 5: Effect of *Bacillus* DY isolate on growth of *Cicer arietinum* (chick pea).

Results

A total of 55 bacterial isolates were obtained of which 40 were from Rhizospheric soil samples and 15 were from dump yard soil samples. CAS plate assay resulted in identification of 4 siderophore producing bacterial isolates. These isolates were designated as RZ-1, RZ-2, RZ-3 and DY. Microscopic and biochemical investigations revealed that RZ-1, RZ-2 and RZ-3 isolates belong to genus *Pseudomonas* and isolate DY was found to belong to genus *Bacillus* RZ-1, RZ-2, RZ-3 and DY include the isolates obtained from rhizospheric and dumpyard soil samples respectively. Among these four isolates, *Bacillus* DY isolate was found to produce maximum zone of chelation (1.5 cm) when inoculated on CAS agar medium. *Pseudomonas* RZ-2, *Pseudomonas* RZ-1 and *Pseudomonas* RZ-3 isolates exhibited 1.00 cm, 0.9 cm and 0.7 cm zones of chelation respectively (Fig. 1 and 2). Culture supernatant from *Bacillus* DY isolate, *Pseudomonas* RZ-2 and *Pseudomonas* RZ-1 exhibited complete discoloration (with in 30 sec) upon addition of CAS reagent. However, culture supernatant from *Pseudomonas* RZ-3 could show only partial decolourization CAS reagent even after 120 sec (Fig. 3). All the four siderophore producers were found to secrete IAA (Fig. 4).

Investigation of effect of incubation time on production of siderophore and IAA revealed that *Bacillus* DY, *Pseudomonas* RZ-2 and *Pseudomonas* RZ-1 isolates secreted siderophores from 16th hr of incubation, *Pseudomonas* RZ-3 exhibited siderophore production at 24th hr of incubation. Among the four isolates, only *Bacillus* DY started IAA production from 16th hr of incubation, other isolates secreted IAA at 24th hr of incubation (Table 1).

The impact of *Bacillus* DY isolate on growth of chickpea plants was studied. This microbial inoculants that produced maximum levels of siderophores and IAA, effectively enhanced chick pea growth. Soil inoculation of *Bacillus* DY resulted in improved root and shoot lengths of chick pea plants compared to control plants (Fig. 5).

Discussion

In this study, some indigenous *Bacillus* and *Pseudomonas* spp. were isolated from dump yard and rhizosphere soils. In the present investigation 3 isolates of *Pseudomonas* sp and 1 isolate belonging to *Bacillus* sp. were screened *in vitro* for PGP activities, all selected bacterial isolates were positive for siderophore production and IAA production. Our findings of IAA production by *Bacillus* and *Pseudomonas* sps are in agreement with the other research findings (Ferreira *et al.*, 2019; Chagas *et al.*, 2015). Pot experiment performed by using *Cicer arietinum* seeds inoculated with PGP *Bacillus* DY isolate significantly enhanced the plant growth by increasing plant root length, plant shoot length, over the uninoculated control. The *Bacterial* isolates are extensively reported in the literature for its PGP potential (Shalini *et al.*, 2019), such isolates are of great significance in plant growth promotion.

Acknowledgment

Authors of this research article sincerely thank Bhavan's Vivekananda College of Science, Humanities & Commerce, Sainikpuri, Secunderabad, Telangana-500094, India for the support provided.

References

Ahmad Humayan Kabir, Nick Paltridge and James Stangoulis (2016). Chlorosis correction and agronomic biofortification in field peas through foliar application of iron fertilizers under Fe deficiency, *Journal of Plant Interactions*, **11(1):** 1-4.

Broadley, M., P. Brown, I. Cakmak, Z. Rengel and F. Zhao (2012). Function of nutrients. In: Marschner P (ed) Marschner's mineral nutrition of higher plants, 3rd edn. Elsevier, Hoboken, pp 191–248.

Bucheli Witschel, M. and T. Egli (2001). Environmental fate and microbial degradation of aminopolycarboxylic acids. *FEMS Microbiol. Rev.*, **25:** 69–106.

Chagas Junior, A.G. De Oliveira1, L.A. De Oliveira, G.R. Dos Santos, L.F.B. Chagas, A.L. Lopes Da Silva and J. Da Luz Costa (2015). Production Of Indole-3-Acetic Acid By Bacillus Isolated From Different Soils**.** *Bulgarian Journal Of Agricultural Science,* **21(2):** 282–287.

Ferreira, C.M.H., A. Vilas-Boas and C.A. Sousa (2019). Comparison of five bacterial strains producing siderophores with ability to chelate iron under alkaline conditions. *AMB Expr.,* **9:** 78.

Gordon, S.A. and R.P. Weber (1951). Colorimetric estimation of indoleacetic acid. *Plant Physiology,* **26:** 192–195.

Harrington, S.A., N. Cobo, M. Karafiátová, J. Doležel, P. Borrill and C. Uauy (2019). Identification of a Dominant Chlorosis Phenotype Through a Forward Screen of the Triticum turgidum *cv.* Kronos TILLING Population. *Front. Plant Sci*., **10:** 963.

Lucena, J.J. (2000). Effects of bicarbonate, nitrate and other environmental factors on iron deficiency chlorosis. A review. *J. Plant Nutr.,* **23:** 1591–1606.

Raaska, L., L. Viikari and T. Mattila-Sandholm (1993). Detection of siderophores in growing cultures of Pseudomonas spp. *J. Ind. Microbiol.,* **11:** 181–186.

Schwyn, B. and J.B. Neilands (1987). Universal chemical assay for the detection and determination of siderophores. *Anal. Biochem.* **160:** 47–56.

Shalini Devi Suvala, Safiya Fatima and Rajini Peddi (2019). Bioprospecting Indigenous Drought Mitigating and Plant Growth-promoting Isolates for Chickpea Cultivation in Telangana, A Semi-Arid Region. *Biopestic. Int.,* **15(2):** 119-127 .