

Original Research Article

<https://doi.org/10.20546/ijcmas.2017.608.227>

Antibiosis of Siderophore Producing Bacterial Isolates against Phytopathogens and Their Effect on Growth of Okra

A. Pahari and B.B. Mishra*

Department of Microbiology, College of Basic Science and Humanities, Orissa University of Agriculture and Technology, Bhubaneswar, India

*Corresponding author

ABSTRACT

Iron is one of the most essential micronutrient for any living cells but the availability of iron is limited due to very low solubility of the dominant ferric iron (Fe^{3+}) in soil. Bacteria can produce low molecular weight iron chelating compound called as siderophore. On account of that, an attempt was made in the present investigation to study the effect of siderophore producing rhizobacteria on growth of Okra and evaluation of biocontrol efficacy of the potential isolates against different phyto-pathogens *in-vitro*. It was observed that the siderophore producing isolates significantly increase the growth parameters like root length, shoot length and biomass of Okra which was also statistically significant and showed antagonistic effect against different phytopathogens including *Rhizoctonia solani* (ITCC-186) and *Fusarium oxysporum* (ITCC-578).

Keywords

Iron,
Rhizobacteria,
Siderophore,
Phyto-pathogens.

Article Info

Accepted:
19 June 2017
Available Online:
10 August 2017

Introduction

The rhizosphere is the narrow region of soil that is directly influenced by root secretions and associated with soil microorganisms. Such bacteria which can grow in the rhizosphere region as well as plant roots and enhance the plant growth are referred as plant growth promoting rhizobacteria (PGPR). Plant Growth Promoting Rhizobacteria promotes growth of plants through direct and indirect mechanisms. In direct mechanism it facilitates nutrient uptake, nitrogen fixation, phosphorus solubilization, siderophore production, IAA and other growth hormone production (Glick, 1995). Indirect mechanisms are control of phytopathogenic microorganisms which are a major and

constant threat to sustainable agriculture and environment stability through production of antibiotics, siderophore, lytic enzymes. Application of Plant growth promoting rhizobacteria is a promising environmental friendly approach to obtain sustainable fertility of the soil and plant growth indirectly.

Iron is an essential micronutrient for almost all living cell because it plays critical role in metabolic processes such as nucleic acid synthesis, respiration and photosynthesis. Despite being most abundant elements in earth's crust, the availability of iron is limited by the very low solubility of Fe^{3+} , the

predominant state of iron in aqueous, non-acidic, oxygenated environments and accumulates in mineral phases such as iron hydroxides as rust, hence cannot be utilized by the plant and due to the imbalance between the solubility of iron in soil and the demand for iron by the plants are the primary causes of iron chlorosis (Thompson and Troeh, 1973; Bodek *et al.*, 1988).

Rhizobacteria have the capability to produce low molecular weight (500-1000 dt) metal chelating compound including iron, called as Siderophore. Siderophore chelate iron from mineral phases by formation of soluble Fe^{3+} complexes and make it available to the plant and enhance the plant (Vansuyt *et al.*, 2007). Generally siderophores are phenolic compound or citric acid derivatives and depending on the oxygen ligands for Fe^{3+} coordination, siderophores can be classified into three main categories i.e. hydroxamates, catecholates, and carboxylates growth (Pahari *et al.*, 2016). More over among the PGPR, siderophore-producing rhizobacteria is the most promising biofertilizer which not only improve the plant iron nutrition but also protect the plant from different types of phytopathogens by chelating iron (Chincholkar *et al.*, 2007b).

Microbiologists have developed techniques for introduction of siderophore producing PGPR in soil system through seed, soil or root applications and enhance plant growth via suppression of phytopathogens. So the use of siderophore producing rhizobacteria as a biofertilizer is one of the best modern tools for agriculture and it is a gift of our modern agricultural science.

On account of that, the present investigation has been undertaken to study the effect of siderophore producing rhizobacteria on growth of Okra and evaluation of biocontrol efficacy of the potential isolates against different phyto-pathogens *in-vitro*.

Materials and Methods

Inoculum preparation for green house study

Previously isolated four different siderophore producing *Bacillus* species i.e. BGBA-1, BGBA-2, BRBA-1 and BRBA-2 (Pahari and Mishra, 2017) were taken from the glycerol stock and streaked onto nutrient agar. Single colony of the bacteria was inoculated and grown in nutrient broth with constant shaking at 150 rpm for 48 h at room temperature. After the incubation period, the cultures were centrifuged at 6000 rpm for 10 min and re-suspended in phosphate buffer (100 mM, pH. 7.0). The cell concentration was adjusted to 9×10^8 cfu/ml. (0.3 OD at 595 nm = 108cfu/ml) (Pahari and Mishra, 2017).

Green house study

Okra seeds (*Abelmoschus esculantus* L.) was collected from the Department of Vegetable Science, College of Agriculture, OUAT for the experiment. For seed treatment, the seeds were surface-sterilized with 0.2% mercuric chloride ($HgCl_2$) for 5 min, and rinsed thoroughly in sterile distilled water for six times air dried for 15 minutes. The seeds were then soaked in 10 ml of the bacterial suspension (10^8 - 10^9 CFU / ml) for 30 minutes. A control was taken with sterile nutrient broth for comparison and after the seeds were air dried (ISTA, 1993; Pahari *et al.*, 2017).

For preparation of sterile soil, field soil was autoclaved twice for 20 min at 120°C with a 24 h interval. Total 2 kg of sterile soil was taken in each pot and watered in soil: water (1:2 w/v). The holes of the pot were closed to prevent of drainage of water. For each treatment, three such pots were maintained. The bacteria treated seeds were showed in soil at 4 to 5 cm depth at the rate of three seeds

per pot and un-inoculated seeds were served as control. Growth parameters like root length, shoot length and biomass were recorded after harvesting of the plant.

Estimation of chlorophyll content

Total chlorophyll content in the leaves were determined by using the method stated by Arnon (1949). For estimation of chlorophyll, the second leaf from the top of 45 days old Okra was taken from all the treatments as well as control and washed with sterile water. After that 100 mg of fresh leaf were cut into small pieces and grinded with 80% acetone (acetone: water, 80:20 v/v) in mortar and pestle. The extract were collected in the test tube and centrifuged at 10,000 rpm for 10 minutes. The absorbance was measured at wavelength of 663nm and 645nm for chlorophyll a and b respectively and the respective chlorophyll content was calculated using the formula and expressed as mg/gm fresh weight leaf.

***In vitro* antagonistic effect of siderophore producing bacteria against different phytopathogens**

The antagonistic effect of siderophore producing bacteria were tested by duel culture method against two common plant pathogen *Rhizoctonia solani* (ITCC-186) and *Fusarium oxysporum* (ITCC-578). Spores of fungal cultures grown on Czapek Dox agar media and a 5 mm diameter mycelial agar disc was cut from the margin of 7-day-old fungus culture and placed on one side of a 9 cm Petri dish containing Czapek Dox agar media. After that test bacteria was streaked on the other end of the Petri dish and incubated at $28 \pm 2^{\circ}\text{C}$ for 5 to 8 days. Dishes inoculated only with test pathogens served as controls. The percent of inhibition of each fungus was measured using the formula (Vincent, 1927): Inhibition percentage (%) = $(R1-R2) / R1 \times$

100 where R1 is radial growth of mycelia in control and R2 is radial growth of mycelia in treatment.

Statistical analysis

All the experiment was done in triplicate and the data was analyzed statistically by one way ANOVA at $p < 0.05$ significant level.

Results and Discussion

In the present investigation it was found that all the siderophore producing rhizobacterial isolates significantly increase the root length, shoot length and biomass of the plant and among them BGBA-1 showed highest activity. Highest root (23.83 cm) and shoot (62.66 cm) elongation was found in Okra when the Okra seeds were treated with BGBA-1 (Table 1). In control the root and shoot length was 13 cm and 35 cm respectively. In case of biomass, maximum root biomass (21.85 gm) and shoot biomass (59.74 gm) gm was observed in BGBA-1 treatment in respect to control. Actually in the soil, plant roots normally coexist with bacteria that may produce siderophores capable of sequestering the available soluble iron, which could interfere with plant growth and function. However, plant roots are sometimes capable of taking up ferric complexes of siderophores and using these as sources of iron (Powell *et al.*, 1982). Siderophore producing bacteria also improve the chlorophyll content in plant because iron was absorbed in ferrous form (Fe^{2+}), which was necessary for the formation of chlorophyll and functions in some enzymes of the plant's respiratory system (Schneider *et al.*, 1968). In the present study, it was found that all the bacterial isolates increase the chlorophyll content of leaf in respect to control. Maximum total chlorophyll i.e. 0.81 mg/gm of fresh leaf was found in BRBA-1 treatment but in control it was 0.28 mg/gm (Table 2).

Table.1 Effect of siderophore producing PGPR isolates on growth of Okra (*Abelmoschus esculentus* L.)

Isolate No.	Root length (cm)	Shoot length (cm)	Biomass of root (gm)	Biomass of shoot (gm)
Control	13.00 ± 0.76	35.00 ± 1.73	10.11± 0.93	27.76 ± 1.08
BGBA-1	23.83 ± 0.72	62.66 ± 2.18	21.85± 1.27	59.74 ± 0.77
BGBA-2	22.00 ± 1.00	54.67 ± 1.76	18.43± 0.39	49.07 ± 1.41
BRBA-1	22.06 ± 1.26	57.66 ± 1.75	21.61± 0.44	52.38 ± 1.12
BRBA-2	19.33 ± 0.88	52.66 ± 2.33	18.52± 0.51	46.38 ± 0.66

Values represents mean ±SE and highly significant at p <0.05

Table.2 Total chlorophyll content (mg/gm) of Okra

Isolate No.	Total Chlorophyll (mg/gm)
Control	0.28 ± 0.04
BGBA-1	0.69 ± 0.03
BGBA-2	0.48 ± 0.01
BRBA-1	0.81 ± 0.02
BRBA-2	0.58 ± 0.01

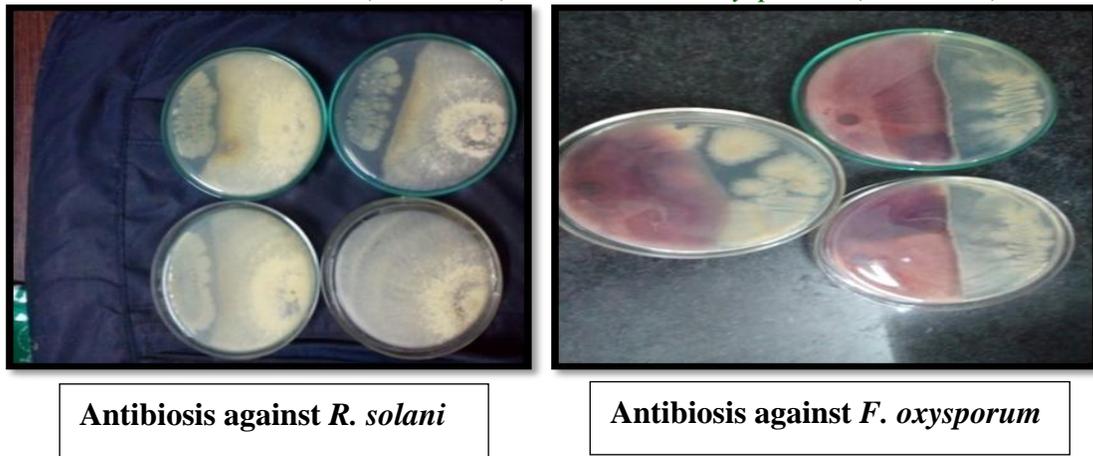
Values represents mean ±SE and highly significant at p <0.05

Table.3 *In vitro* antagonistic effect of siderophore producing bacteria on mycelial growth of *Rhizoctonia solani* (ITCC 186) and *Fusarium oxysporum* (ITCC 578)

Isolate No.	Inhibition %	
	<i>Rhizoctonia solani</i>	<i>Fusarium oxysporum</i>
BGBA-1	47.91± 1.10	55.17 ± 0.65
BGBA-2	38.33± 1.10	41.16 ± 0.72
BRBA-1	51.08± 2.08	52.27 ± 0.74
BRBA-2	46.67 ± 1.81	50.00 ± 0.86

Values represents mean ±SE and highly significant at p <0.05

Fig.1 *In vitro* antagonistic effect of siderophore producing bacteria on mycelial growth of *Rhizoctonia solani* (ITCC 186) and *Fusarium oxysporum* (ITCC 578)



The antagonistic effect of the isolates was also checked against two common phytopathogens

by duel culture method. Maximum mycelial inhibition 51.02% was found against *R. solani*

by BRBA-1 and in case *F. oxysporum*, it was 55.17% by BGBA-1 (Table 3 and Fig. 1).

It was already proved that siderophore producing bacteria having plant growth promoting function can protect the plant from different types of plant pathogens (Raupach and Kloepper, 1998; Chincholkar *et al.*, 2007b). So from the present study it is concluded that application of siderophore producing rhizobacteria by seed bacterization is a novel approach to replace chemical fertilizers and pesticides for sustainable agriculture in India.

Acknowledgement

The authors are thankful to the Staff of the Department of Vegetable science, College of Agriculture, OUAT for providing laboratory facilities during the period of study. The authors have no conflict of interest to declare.

References

Arnon, D.I. 1949. Copper enzymes in isolated chloroplasts, polyphenoloxidase in *Beta vulgaris*. *Plant Physiol.*, 24: 1-15.

Bodek. I., Lyman, W.J., Reehl, W.F. and Rosenblatt, D.H. 1988. Environmental Inorganic Chemistry: Properties, Processes, and Estimation Methods. SETAC Special Publication Series, B.T. Walton and R.A. Conway, editors. *Pergamon Press*. New York.

Chincholkar, S.B., Chaudhari, B.L., Rane, M.R. and Sarode, P.D. 2007b. Fungal phytopathogen suppression using siderophoregenic bio-inoculants. In: *Biological Control of Plant Diseases: Current Concepts*. Chincholkar, S. B. and Mukerji, K.G. (Eds). Haworth Press, USA. pp. 401-417.

Glick, B.R. 1995. The enhancement of plant growth by free-living bacteria. *Can. J. Microbiol.*, 41: 109-117.

ISTA. 1993. Proceedings of the international Seed Testing Association, International Rules for Seed Testing. *Seed Science Technology*. 21: 25-30.

Pahari, A., Dangar, T.K. and Mishra, B.B. 2016. Siderophore quantification of bacteria from sundarban and its effect on growth of brinjal (*Solanum melongena* L.). *The Bioscan* 11(4): 2147-2151.

Pahari, A., Pradhan, A., Maity, S. and Mishra, B.B. 2017. Carrier Based Formulation of Plant Growth Promoting *Bacillus* Species and their Effect on Different Crop Plants. *Int.J.Curr.Microbiol.App.Sci.* 6(5): 379-385

Pahari, A. and Mishra, B.B. 2017. Characterization of Siderophore Producing Rhizobacteria and Its Effect on Growth Performance of Different Vegetables. *Int.J.Curr.Microbiol.App.Sci.* 6(5): 1398-1405.

Powell, P.E., Szaniszlo, P.J., Cline, G.R. and Reid, C.P.P. 1982. Hydroxamate siderophores in the iron nutrition of plant. *J. Plant Nutri*, 5: 653-673.

Raupach, G.S. and Kloepper, J.W. 1998. Mixtures of plant growth promoting rhizobacteria enhance biological control of multiple cucumber pathogens. *Phytopathol*, 88: 1158– 1164.

Schneider EL, Loke SP and Hopkins DT. 1968. GLC analysis of cyclopropenoid acids. *JAOCS*, 45: 585 – 590.

Thompson, L.M. and Troeh, F.R. 1973. *Soils and Soil Fertility*, third ed. McGraw-Hill Book Company.

Vansuyt, G., Robin. A., Briat, J.F., Curie, C. and Lemanceau. P. 2007. Iron acquisition from Fe-pyoverdine by *Arabidopsis thaliana*. *Molecular Plant-Microbe Interactions*, 20: 441-447.

Vincent, J.M. 1927. Distortion of fungal hyphae in presence of certation inhibitors. *Nature*, 59: 850.

How to cite this article:

Pahari, A. and Mishra, B.B. 2017. Antibiosis of Siderophore Producing Bacterial Isolates against Phytopathogens and Their Effect on Growth of Okra. *Int.J.Curr.Microbiol.App.Sci.* 6(8): 1925-1929. doi: <https://doi.org/10.20546/ijcmas.2017.608.227>