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# EVALUATION OF THE BIOCONTROL EFFICACY OF SIDEROPHORE PRODUCED FROM AGRICULTURAL ISOLATES AGAINST SOME FUNGAL PHYTOPATHOGENS

**Kamble N.P.*** Department of Microbiology, Badrinarayan Barwale Mahavidyalaya, Jalna
*Corresponding Author

**Naphade B. S.** Department of Microbiology, Badrinarayan Barwale Mahavidyalaya, Jalna

**ABSTRACT** Plant growth promoting bacteria play an important role in agriculture by promoting beneficial effect, it gives novel and potential tool to replace chemical fertilizer, pesticides and supplements. The aim of the present study was to evaluate biocontrol efficacy of plant growth promoting bacteria isolated from agricultural soil of Pune and Aurangabad district. In present study ten plant growth promoting bacteria were isolated and selected and screened in vitro for detecting plant growth promoting traits like production of indole acetic acid (IAA), Ammonia, HCN, Siderophore, Phosphate solubilization and also evaluated for biocontrol efficacy of siderophores produced by isolated bacteria against some fungal phytopathogens such as *A .nigar, Fusarium oxysporum, Curvularia sp. Helminthosporium sp. Colletotrichum* and *A.flavous.* Antifugal activity of Siderophore was determined by agar well diffusion method. It was observed that four isolates namely V1, P1, P3 and L2 showed the significant production of Siderophores and showed potential biocontrol agent against fungal phytopathogens. Siderophores are useful in plant growth improvement and disease management. Agricultural microbiologists have interests in using beneficial microbes as a solution for the over use of potentially harmful chemical fertilizer and pesticides.

**KEYWORDS :** Fungal pathogens, Biocontrol activity, Siderophore

## INTRODUCTION

Fungal plant pathogens are the one of important factor that cause heavy loss to agricultural product commonly (Ekundaya *et al.,* 2011). PGPR's control a wide range of phytopathogens like fungi, bacteria, viruses, nematodes, etc., by competition or antagonism mechanisms, however, the most studied phenomenon is the induction of systemic resistance by these bacteria in the host plant. (Rohit Kumar *et al* 2011) Biological control has become effective strategy for management of disease which is environment friendly and ecology conscious (Kamala Devi 2012). The increasing use of chemicals as pesticides to eliminate plant pathogens has provided effective solutions in agriculture. However, due to the fact that the excessive use of these chemicals such as thiabendazole and o-phenylphenol causes environmental pollution, and as plant pathogenic agents are quickly become resistant to chemical pesticides and considering the high price of pesticides and their accumulation in plants or soil which has harmful effects on humans, extensive researches are being conducted in the world to replace this method with more recent methods to confront fungicidal resistant pathogens. Since the late 1900s, scientists have made great efforts to use natural antagonisms of terricolous microorganisms to protect plants. Some bacteria exhibit activities against pathogens such as pathogenic fungi due to the ability to produce anti-microbial compounds such as antifungal lipopeptides and some antibiotics (Mavrodi *et al.,* 2017).

PGPR bacteria exert beneficial effect on plant directly or indirectly. Direct effect includes production of metabolite such as auxins, cytokinine, gibberellins and solublization of phosphate and indirect effect include removal of pathogen by production of secondary metabolites such as hydrogen cyanide and siderophores (Noori *et al* 2012). PGPR have ability to colonizing root system and improve plant growth by suppressing establishment of plant pathogen (Kloepper 2003 and Glick 1995). PGPR contribute in plant growth by secreting certain substances which targets the fungi. Some bacteria like *P. fluroscens* can produce antifungal antibiotics like 2,4-diacetylphloroglucinol whereas others can degrade fusaric acid which is the causative agent of wilting observed in plants. Some can produce enzymes that lyse the fungal cells like *P. stutzeri* produces chitinases (Nowak *et al.,* 1994, Kumar *et al.,* 2002). Siderophores are low molecular weight compounds and chelae with ferric iron Fe3+ with high specific activity and serve as vehicle for the transport fe (III) into microbial cell. It varies in chemical structure, most have either hydroxamate or catechol group that are involved in iron (III) chelation. Transportation of iron is mediated by specific membrane receptor and transport system that recognized the iron siderophore complex (Joyce *et al* 1990). The importance of siderophore is closely related to iron which is an essential element for different biological processes (Anelise *et al*., 2012). These compounds are secreted by microorganisms under low iron stress. It has potential of being used as biocontrol agent by sequestering iron from pathogen thus limiting their growth. (Prema and Selvarani 2013). Siderophore plays the central role in determining the ability of different microorganisms to improve plant development and are also important in iron uptake by a plant even in the presence of other metals such as nickel and cadmium (Burd *et al.,* 1998, Dimkpa *et al.,* 2008). Siderophores are useful in plant growth improvement and disease management. Agricultural microbiologists have interests in using beneficial microbes as a solution for the overuse of potentially harmful pesticides (Patil *et al.,* 2014). Therefore, the present study is focused to examine the siderophore production and their biocontrol properties against some fungal phytopathogens activity

## MATERIALS AND METHODS

**Collection of Soil samples:** Soil Samples were collected from Rhizosphere region of plants from different sites of Maharashtra. Samples were carefully collected in sterilized zipped plastic bags and stored at $4^0$C.

**Phosphate solubilization:** The screening of isolates for phosphate solubilization was done by spot inoculation of isolate on Pikovskaya's agar plate (Subbarao method 1999) Which is inoculated at $30^0$C for 3 to 4 days. After incubation clear zones were observed in the vicinity of the colonies which is an indicator for positive Phosphate solubilization.

**Siderophore production:** Siderophore production was checked on the chrome azurole S agar (CAS) plates described by Alexander D.B.and D.A. Zuberer. Spot inoculation of the test culture was done on the plates and were incubated at $30^0$C for 5 days. Appearance of yellow-orange hallo zone around the colony indicates positive result.

**IAA Production:** IAA Production by isolates was tested by using nutrient broth containing 0.1 % DL tryptophan inoculated with isolate and incubated at $30^0$ C for 48 hrs. On orbital shaker at 150 rpm. After incubation Salkowaski method was used to determined IAA production calorimetrically (Gordon and Weber 1951).

**Ammonia Production:** Production of Ammonia was determined by Cappuccino and Sherman method. 10 ml peptone broth was inoculated with isolate and incubated at $30^0$ C for 48 hrs on orbital shaker at 120 rpm. 0.5 ml Nessler's reagent was added to each tube after development of faint yellow to brown color indicating the production of ammonia.

**HCN Production:** HCN Production by isolate tested by using methodology described by Castric Isolate streaked on nutrient agar medium containing 4.4g per liter of glycine Whatman filter paper No.1 soaked in 0.5 % picric acid solution containing 2% sodium carbonate was placed inside the lid of plate. Plates were sealed with parafilm and incubated at $30^0$C for 4 days. After incubation development of light brown to dark brown colour spots indicates HCN Production

**In vitro Antifungal activity:** Antifungal activity of Siderophore was determined by agar well diffusion method described by Ahmad *et al.,* (1999).Test fungal cultures grown on potato dextrose agar (PDA), 0.1ml of normal saline suspension of fungal spore was spread on PDA plates and well of 8mm diameter were punched into agar medium and filled with 200 µl of cell free culture supernatant contain siderophore produced in succinate media Previously Siderophore presence was checked by using CAS assay (Payne 1994). Plates was incubated for 5 days at 27 ± 2 °C. Bacterial antifungal activity was determined by zone of inhibition in mm.

## RESULTS AND DISCUSSION

Agriculture play major role in economic stability of India. Microbial inoculants can act as an alternative to chemical fertilizer and fungicides and has gained importance due to increased concerns on environmental pollution, pathogen resistance and high plant protection costs. Phosphate one of major limiting nutrient for plant growth Phosphate present in soil in two insoluble forms thus solublization and mineralization of phosphate by phosphate solubilizing bacteria (PSB) is one of the most important bacteria physiological traits in soil biogeochemical cycles (Jeffries et al.,2003) In the present study isolated Plant growth promoting bacteria from Agricultural soil collected from different locations of Pune and Aurangabad district **were subjected for** screening for different Plant Growth Promotion traits such as Phosphate solubilization, IAA production, siderophore production, Ammonia production and HCN production. All isolates showed maximum plant growth promotion activity. Production of phytohormones or growth regulators is most effective direct mechanism of PGPR for plant growth promotion. (Glick 1995) Natural compound such as indole possessing plant growth promoting activity are called as auxins Growth regulators like auxins regulate most of the physiological activities and growth in the plants. Antifungal metabolites like HCN produced by PGPR play important role in antifungal activity. Economic stability of India is dependent on the agricultural field.

Research work in the last 50 yrs by agricultural scientists has its major thrust on increasing crop productivity. Such microbial metabolites can act as an alternative to chemical fungicides and has gained importance due to increased concerns on environmental pollution, pathogen resistance and high plant protection costs (Angel *et al* ,2013).Siderophores, bacteriocins and antibiotics are three of the most effective and well known mechanisms that an antagonist can use to limit phytopathogenic proliferation (Anelise *et al*, 2012). Cultures were isolated from Agricultural soil collected from different locations of Pune and Aurangabad district**.** Plant Growth Promotion traits of four P1, P2, L2, V3 isolates such as Phosphate Solublization, IAA production, Siderophore production, Ammonia production and HCN production were identified. P1 and L2 show presence of all these traits were as p2 and v3 are show maximum pgpr activity except HCN production. All the isolates have good Pgpr activity All the isolates were Siderophore positive further use to demonstrate their potential as biocontrol agent. Zone of fungal growth inhibition is found around the siderophore caring well More than 50% biocontrol activity showed by these isolates against fungal cultures tested. control has no growth inhibition. (Table 1) Each isolate showed biocontrol activity against more than 50% fungal cultures of total fungal cultures tested. There was no growth inhibition in any of the controls kept (Fig.1).

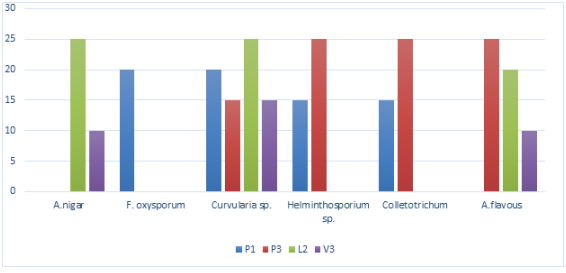
In many instances a correlation has been established between antagonist in vitro and protection in the field (Pal and Gardner 2006). The present studies serve of siderophore producing bacteria as a biocontrol agent is a good indication. But further study is necessary to find out solutions to various problems encountered in the field of agriculture.

**Table 1. Antifungal activity of Siderophore producing Bacterial isolates (Zone of inhibition in mm)**

| **Fungal pathogens** | *A.nigar* | *F. oxysporum* | *Curvularia sp.* | *Helminthosporium sp.* | *Colletotrichum* | *A.flavous* |
|---|---|---|---|---|---|---|
| **P1** | **-** | **20** | **20** | **15** | **15** | |
| **P3** | | **-** | **15** | **25** | **25** | **25** |
| **L2** | **25** | **-** | **25** | **-** | **-** | **20** |
| **V3** | **10** | **-** | **15** | **-** | **-** | **10** |

(-)= no inhibition

**Fig 1: Antifungal activity of Siderophore against fungal phytopathogens**




## CONCLUSION:

The results of the present study, thus, indicate the potential of harnessing the benefit of plant growth promoting as biocontrol agents against some fungal phytopathogens. Siderophores are useful in plant growth improvement and disease management. Agricultural microbiologists have interests in using beneficial microbes as a solution for the over use of potentially harmful chemical fertilizer and pesticides.

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