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Research Article

In vitro screening of Agriculture soil isolates for plant growth promotion traits

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Abstract

Plant growth promoting rhizobacteria (PGPR) are environmental friendly and offer sustainable approach to increase production of crops and health.In the present study, isolates from ten agriculture soil were collected from different locations of Pune and Aurangabad district. *Invitro* screening was done for different Plant Growth Promotion activities such as Phosphate solublization, IAA production, siderophore production, Ammonia production and HCN production. All isolates were found positive for phosphate solublization, IAA production, Ammonia production and catalase and only two isolates were showing positive test for HCN production. All isolates showed maximum plant growth promotion activity. Therefore these isolates can be utilized as a biofertilizer formulation under local agroclimate conditions of Pune and Aurangabad.

INTRODUCTION

Rhizosphere provides environment for the growth of active microbes which have capability of exerting beneficial nutrient, detrimental effect on plant growth. Rhizobacterial or root colonizing bacteria shows direct effect on the growth of host plant via direct or indirect mechanism and hence termed as Plant growth promoting bacteria (Juanda, 2005) Through mechanism by which PGPR promote plant growth are not yet completely clear. But many different traits of these bacteria attribute for growth promotion activity (Cattelan *et al.*,1999)

PGPR may have the ability to produce a change in the concentration of plant hormones like IAA, Gibberellin ,cytokine, ethylene, fixation of nitrogen.Suppress growth of harmful microorganism by production of siderophore, β-1,3 glucanase,chitinase,antibiotics and cyanide. (Kennedy, 1998). Apart from nitrogen fixation, phosphate solublization is an important plant growth promoting activity, soluble inorganic phosphate added to soil get immobilized and not available to plants (Rodriguez, 1999). Availability of phosphorus to plants is influenced by microorganisms through the process of mineralization and demineralization. Many microorganisms which are abundant on root surfaces of plants can solubilize phosphorus that is not available to plants (Jadhav, 2013).

Growth regulators like auxins regulate most of the physiological activities and growth in the plants. Natural compound such as indole possessing plant growth promoting activity are called as auxins. Plant associated bacteria are usually found to have the ability of producing phytohormones (Halda-Alija, 2003). Auxin, chemically called as indole acetic acid plays a major role in plant growth and development as a regulator of numerous biological processes, from cell division, elongation and differentiation to tropic responses, fruit development and senescence. Role of phytohormone biosynthesis by microorganisms is not fully elucidated but it was indicated that there might exist a symbiotic association between plants

and microorganisms (Normanly *et al*., 1993). Plants and microorganisms synthesize auxins by the different the biosynthetic pathways, depending on the plant and/or microorganism. Auxins can be produced by more than 80% of soil bacteria in the rhizosphere and hence, these microorganisms have ability to affect the endogenous levels of this regulator and, there are fore, these have remarkable effects on plant growth (Jha, 2015). However, microbially produced phytohormones are more effective due to the reason that the threshold between inhibitory and stimulatory levels of chemically produced hormones is low, while microbial hormones are more effective by virtue of their continuous slow release (Gupta, 2015). Microrobial auxin production is the major factor responsible for strengthening the plant-microbe relationship and it also promotes plant growth and its overall development positively. Thus, bacterial auxin production potential can be utilized for plant growth improvement which in turn may be effective in reducing the detrimental effects of chemical fertilizers on the ecosystem (Ahmed, 2014). Auxins are produced in one tissue (shoot) and migrate to effect the development of another tissue (Gopale, 2011).

All living organism require iron as important element for many cellular processes as cofactor and for electron transport chain (Litwin and Calder wood, 1993). Aerobic microorganisms need iron for reduction of oxygen for synthesis of ATP, for haeme formation and many other essential purposes. The planet has aerobic atmosphere which cause surface iron to oxidize to insoluble oxyhydroxide polymer which in turn reduces available free iron level to situation further for iron acquisition microorganism adapted a way by producing iron chelating molecule that is siderophore. Siderophore is a low molecular weight compound produced by many bacteria. It forms complex with free iron and gets transported into the cell through membrane receptor molecules. It also shows virulence mechanisms against plant and animal pathogens. (Glick *et al*., 1999, Loper *et al*., 1999, Neilands, 1981, Lewin, 1984).

There are many literaturesources which describe that plant associated bacteria can be use potentially as agents which stimulate plant growth and help to manage soil and plant health.(Sajid Shaikh *et al* ., 2015*)*. Several soil bacteria play important role in plant growth promotion for e.g. *Pseudomonas, Azospirillum, Azotobacter, Klebsilla, Enterobacter, Alcaligenes, Arthobacter, Burkholderia, Bacillus, Serratia.* (Saharan BS and Nehra V, 2011) PGPR microbial inoculants have been reported to give promising group yield, hence such inoculants can serve as Biofertilizers that can improve soil fertility and crop productivity.

MATERIALS AND METHODS

Soil Sampling

Soil Samples were collected from Rhizosphere region of plants from different sites (Satara, Wai, Pune, Aurangabad, Jalna) of Maharashtra.Samples were carefully collected in zipped sterilized plastic bags and stored at 4^0 C.

Phosphate solublization

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.andD.A.Zuberer (1991) Spot inoculation of the test culture was done on the plates and were incubated at 30^0C 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 colorimetrically (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 hrson 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 theproduction of ammonia.

HCN Production

HCN Production by isolate tested by using methodology described by Castric (1975) Isolate streaked on nutrient agar medium containing 4.4g per liter of glycine Whatmann 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 30^0C for 4 days. After incubation development of light brown to dark brown color spots indicates HCN Production

RESULTS AND DISCUSSION

Cultures were isolated from the rhizospheric region of the soil collected from different regions of Maharashtra. In present study ten cultures were selected and screened for plant growth promoting activity. All the cultures showed the maximum plant growth promoting traits (summarized in table-1).

PGPRcolonize plant roots and exert beneficial effect on plant growth and development. Several hypotheses by which PGPR stimulate plant growth are postulated, although the exact mechanism is not known. Production of phytohormones, suppression of deleterious organisms, activation of phosphate solubilization and promotion of mineral nutrients uptake are usually believed to be involved. So, the common PGPR traits are considered to be auxin synthesis siderophore production, phosphate solubilization, HCN and ammonia production.

Table 1: Screening of soil bacteria for PGPR activity.

Bacterial isolates	Phosphate solubilization	Siderophore production	IAA production	Ammonia production	HCN
P1 (Pune)	+	+	+	+	+
P2 (Pune)	+	+	+	+	-
P3 (Pune)	+	+	+	+	-
L1 (Satara)	+	-	+	+	-
L2 (Satara)	+	+	+	+	+
L3 (Satara)	+	-	+	+	-
J5 (Jalna)	+	+	+	+	-
N1 (Aurangabad)	+	+	+	+	-
A1 (Aurangabad)	+	-	+	+	-
V3 (Wai)	+	+	+	+	-

Key : + = Positive, - = Negative

Phosphate is essential major nutrient required by plant and most of it is present in insoluble form (Nautiyal, 1999). The ability of bacteria to solubilize mineral phosphate and make it available to plant as it enhances the plant growth is a topic of interest to agricultural microbiologist

Phosphate solubilization ability of bacteria can be detected by using agar plate method. All the isolate showing clear zone around the colony were able to solublize phosphate.

Iron is essential requirement of plant and microorganism. Siderophore producing bacteria make it available to plant and also these bacteria competefor iron with soil borne pathogen and play a role as biocontrol agent (Hofte *et al*., 1992, Loper and Henkels, 1997) Out of ten isolates, seven isolates were positive for siderophore production that shows yellow orange zone around the colony.

Isolates from rhizospheres are more efficient auxin produced than isolates from bulk soil (Glick and Bashan, 1997).

IAA production was found to be a common trait in all isolates. All isolate were positive for IAA production that shows pink color formation after addition of Salkowski reagent in 48hrs grown broth. Another important trait of PGPR is Ammonia production that indirectly influence the plant growth. This was also common traits observed in all isolates.

Bacteria like Pseudomonas exert beneficial effect on plant by production of metabolite like HCN. It control the growth of root rot pathogen (Deshwal and Kumar, 2013). Only two isolates were found to show HCN production.

Hence, it is concluded that the isolated bacteria can be efficiently used in agricultural soils and have the potential in future to be exploited in biofertilizer formulations.

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