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MICROBIOLOGY

Biosynthesis of Phytohormones by Potassium Solubilising Bacteria Isolated from Banana Rhizosphere

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ABSTRACT

Phytohormones regulate plant growth in different stages of plants and occur in very low concentrations. Phytohormones are mainly signal molecules involved in cell elongation, apical dominance, tissue differentiation and cell division. Phytohormones can induce several reactions and based on the reactions they are divided into auxin, cytokinin , gibberellin , ethylene and abscisic acid. Production of phytohormones are not only associated with plants, occurrence of phytohormones are also involved with microorganisms mainly beneficial rhizobacteria. Rhizosphere microorganisms are naturally occurring beneficial bacteria which colonize the plant roots and promotes growth by enhancing nutrient availability and producing plant growth promoting substances. Potassium solubilizing bacteria are also rhizospheric microorganisms which produce different phytohormones. The present study has taken up to investigate the production of phytohormones by the potassium solubilizing bacteria isolated from banana rhizosphere. All the six isolates showed good amount of phytohormone production. Among the six KSB isolates, isolate SAF showed highest amount of cytokinin production, whereas SAM showed maximum content of gibberelic acid and SBF showed maximum IAA production. Hence, the present study indicates that potassium solubilizing bacteria which solubilizes insoluble potassium from the soil also have the capacity to produce phytohormones which can promote plant growth.

Highlights

- KSB isolate SAF produced highest amount of cytokinin, isolate SAM produced highest amount of gibberelic acid and SBF produced highest IAA.
- KSB isolates can be recommended as a potential biofertilizer to replace chemical fertilizers.

Keywords: Potassium solubilizing bacteria, biofertilizers, phytohormones, growth promotion, PGPR

Potassium is the third essential macronutrient essential for plant growth and the most abundantly absorbed cation in higher plants. As more than 90 per cent of potassium exists in the form of insoluble rock and silicate minerals, the concentration of soluble potassium is usually very low in soil (Parmar and Sindhu 2013). As Potassium is found in fixed forms in soil and are available in very low amount to the plants and at the same time excessive use of chemical fertilizers causing the depletion of potassium from potassium reserve in faster rate. As a consequence, potassium deficiency is becoming one of the major constraints in crop production. Deficiency of potassium can lead to plants with poorly developed roots, low seed production, slow growth rate, and a lower yield. So, there is an essential need to find an alternative source of potassium for maintaining potassium status and plant uptake in soils for sustaining crop production (Kumar and Dubey 2012). Potassium solubilizing bacteria which is of rhizosphere origin has the ability to solubilize insoluble form of potassium by following different mechanism like production of organic acids, siderophores have been reported. Potassium solubilizing rhizobacteria such as such as *Acidothio bacillus* sp., *Bacillus edaphicus*,

Ferrooxidans sp., *Bacillus mucilaginosus*, *Pseudomonas* sp., *Burkholderia* sp., and *Paenibacillus* sp. have been reported to release potassium in accessible form from potassium-bearing minerals in soils (Liu *et al*. 2012). Application of potassium solubilizing rhizobacteria can improve crop production and can reduce the use of synthetic fertilizers and support eco-friendly crop production (Setiawati and Mutmainnah 2016).

Rhizosphere microorganisms have diverse beneficial effects on plants through different mechanisms like nitrogen fixation and nodulation (Raza *et al*. 2016), phosphate solubilization (Ahemad and Khan 2012) production of indole-3-acetic acid (IAA), siderophores (Jahanian *et al*. 2012), degradation of environmental pollutants, and production of hormones and antibiotics or lytic enzymes (Xie *et al*. 2016).

Phytohormones or plant growth regulators are synthetic substances produced by plants, regulates germination, growth metabolism, and other physiological activities mainly functions as chemical messenger for intercellular communication are required in small amount. It promotes and influence the growth, development, and differentiation of cells and tissues (Opik *et al.* 2005). There are five major groups of plant hormones: auxins, gibberellins, cytokinins, abscisic acid (ABA) and ethylene. Auxins are associated with cell elongation, root initiation , apical dominance and vascular tissue development etc The most common form of auxin found in plants are Indole aceteic acid (IAA). Cytokinins are involved in inducing shoot bud formation and stimulates cell division. Gibberelins initiates flowering , elongation of stem, mobilization of storage materials. Phytohormones are generally produced by plants, but plant growth promoting micoorganisms are also involved in releasing the phytohormones and induces growth and development. Hence, the present study has taken up to investigate efficacy of phytohormone production by potassium solubilizing rhizobacteria isloated from banana rhizosphere.

MATERIALS AND METHODS

The present study was conducted at Department of Agricultural Microbiology, UAS, GKVK, Bengaluru. The potassium solubilizing isolates were isolated from banana rhizosphere soil amended with two different insoluble potassium source. All the KSB isolates were regularly sub cultured and maintained on Aleksandrov's medium.

Six potassium solubilizing isolates were investigated for the production of Auxin, cytokinin and gibberelic acid in broth culture.

Determination of IAA production by cucumber root elongation test

Cucumber seeds were taken for root elongation test for the production of IAA. The seeds were soaked in distilled water and germinated for 6 hours in petriplates in dark condition. Ten days old culture was taken and centrifuged at 5000 rpm for 10 minutes. Germinated seeds were then placed on the petriplates containing filter paper and 1 ml of supernatant was inoculated and the seeds were incubated. After 24 hour of incubation root length was taken and the standard graph was drawn.

Determination of IAA production by KSB isolates (L-Tryptophan method)

For IAA production, the culture medium was inoculated with ten day old culture of KSB isolates. The IAA production medium consisted of peptone 10 g/L, yeast extract 3 g/L, tryptone 0.5%, tryptophan 5 g/L, Agar 15 g/L and the pH was adjusted between 6.8 and 7.0 pH. The cultures were inoculated in 50 mL of above medium in 100 mL flasks and were incubated at 37°C, 160 rpm for 72 h for 10 days. 5 mL medium was withdrawn after 10 days and was centrifuged at 11000 rpm for 15 mins. The experiment was conducted in triplicates. The medium was assayed using Salkovasky's (50 mL of 35% perchloric acid and 1 mL 0.5 M FeCl3 solution) reagent by incubating it in dark for 1 h (Sarwar and Kremer 1995). Development of pink coloration was measured at 530 nm using UV Vis Spectrophotometer.

Production of Cytokinins by KSB isolates

Cucumber seeds were taken and germinated for 3 days in the dark condition. After germination, the initial weight of the cotyledons were recorded and placed in the petriplates. 10 day old culture isolate was centrifuged at 9000 rpm for 5 minutes and 5 ml of the supernatant were inoculated to the cotyledons. The cotyledons were incubated for 24

hours and exposed to fluorescent light for 6 hours and the final weight was taken. The increased in weight was noted.

The cotyledons were then transferred to the test tubes containing 2:1 ratio of Acetone: Alcohol and kept under dark condition for 24 hours. Absorbance was taken at 649 and 663 nm by using UV Vis Spectrophotometer and the standard graph was drawn.

Bioassay of Gibberelic Acid produced by KSB Isolates

Determination of GA3 production by the formation of halo zone

To determine the production of gibberellic acid, paddy seeds were soaked in sterile distilled water for 24 hours. Five paddy seeds were placed per petriplate. Ten day old culture isolate was centrifuged at 5000 rpm for 10 minutes and 5 ml of supernatant was transferred and incubated for 24 hours. Paddy seeds were cut into two halves exactly at the centre and were transferred to starch agar plate. After 24 hours of incubation starch agar plates were flooded with Gram's iodine solution. Halo zone was observed and the measurement of the diameter recorded.

Determination of GA3 production

Gibberellic acid production of KSB isolates were carried out in nutrient medium. Culture suspension was added to the nutrient medium , was incubated at 37 °C for 5 days and was centrifuged at 10,000 rpm for 20 min. The pH of the supernatant was adjusted at 2.5 by using 15% HCL. The filtrate was extracted with ethyl acetate (1:3 of filtrate is to solvent ratio) and the extract was used for its gibberellic acid determination. The experiment was conducted in triplicates. In this assay, gibberellic acid is converted into gibberellenic acid and is estimated at 254 nm absorbance (Pandya and Desai 2014).

RESULTS AND DISCUSSION

Determination of Cytokinins produced by KSB isolates

The data in table 1 shows the results of cytokinins. All the six KSB isolates produced cytokinins and showed increase in weight of cotyledons. Highest increase in weight of cotyledons and cytokinin content were observed in SAF. Other KSB isolates also produced good amount of cytokinin. Increase in weight of cotyledons indicates that KSB isolates might have produced growth promoting substance which has resulted in increase in weight of the cotyledons.

Table 1: Enhancement of cotyledon growth and production of Cytokinin by KSB Isolates

Isolates	Initial weight of the cotyledon (mm)	Final weight of the cotyledon (mm)	Weight increased (mm)	Cytokinin content (µg/ml)
SAM	0.206	0.447	0.241	15.08
SCM	0.417	0.660	0.243	14.38
SDM	0.196	0.446	0.250	13.25
SAF	0.130	0.417	**0.287**	**17.37**
SBF	0.405	0.239	0.166	16.06
SCF	0.347	0.530	0.183	15.24
CD @ 5%	0.01	0.02	0.01	0.21
SEM±	0.00	0.01	0.00	0.07

Cytokinin production in several rhizobacteria has been well studied by many researchers. *Pseudomonas*, *Azospirillum*, and *Bacillus* have been reported to possess the ability to produce cytokinins (Maheshwari *et al*. 2015).

Determination of IAA produced by KSB isolates

A large number of rhizobacteria produce auxin, a plant growth hormone, which has a role in plant growth and development. Research has demonstrated that auxin producing bacteria can be successfully used in increasing crop yield (Javed and Arshad 1999). Tryptophan is a major precursor for auxin production by many of rhizobacteria (Kamilova *et al*. 2006). Different rhizobacteria possess different routes for the synthesis of IAA. IAA is synthesized by plant-associated microbes via L-tryptophan-dependent and independent pathways. Most of these rhizobacteria utilize L-tryptophan which is secreted in root exudates as a precursor for IAA production.

The results pertaining to the production of IAA were presented in Table 1. The results revealed

that all the six KSB isolates were found to produce reasonable amount of IAA. Isolate SBF and SCM showed highest IAA production. The least IAA production was showed in SAF.

Table 2: Production of IndoleAceticAcid (IAA) by KSB Isolates

Isolates	Root length (cm)	IAA (µg/ml) (Direct method)	IAA (µg/ml) (L-tryptophan method)
SAM	5.04	17.44	126.50
SCM	5.59	20.54	152.00
SDM	4.74	18.52	128.00
SAF	4.12	15.13	81.00
SBF	**6.39**	**23.49**	**164.00**
SCF	5.10	18.74	87.50
CD @ 5%	0.07	0.27	1.92
SEM±	0.02	0.09	0.64

The data in table 2 reveals that elongation of root length was induced by the production of IAA. Maximum root elongation was observed in SBF followed by SCM. The results are in the conformity of the findings of Jha & Saraf (2015) and Spaepen *et al*. (2007). They found that *Azospirillum brasilense* produce IAA by utilizing L-tryptophan.

Indole-3-acetic acid, as the main auxin form, plays important role in formation of plant tissues. IAA enhances meristematic activity causing root cells division thereby contributing to enhanced root growth (Friml, 2003). IAA can also affect root hair elongation (Rahman *et al.* 2002) and root formation (Pitts *et al.*, 1998) specially when Tryptophan is present (Husen 2003).Therefore, research has demonstrated that auxin producing bacteria can be successfully used in increasing crop yield (Javed and Arshad, 1999).

Determination of GA3 produced by KSB isolates

Gibberellic acid produced by KSB isolates were given in Table 3. All the six KSB isolates produced good amount of gibberellic acid. Halo zone was observed maximum in isolate SAM followed by SDM and SCF. Gibberellic acid production was also observed highest in SAM.

Present study are supported by the findings of Desai (2017) and Ambawade and Pathade (2013). They also found that significant production of gibberellic acid was observed with the rhizobacteria *Pseudomonas*, *Azotobacter* and *Bacillus* respectively.

Table 3: Production of Gibberellic acid by KSB isolates

Isolates	Diameter of the Zone (mm)	GA (µg/ml)
SAM	**12.00**	**3.99**
SCM	8.20	2.73
SDM	9.70	3.23
SAF	7.40	2.46
SBF	7.60	2.53
SCF	9.60	3.19
CD @ 5%	0.33	0.04
SEM±	0.11	0.01

CONCLUSION

The present study has focused on the production of various phytohormones such as IAA, cytokinin and gibberellic acid by the potassium solubilizing bacteria, a rhizospheric microorganism isolated from banana rhizosphere soil. Three isolates SAM, SAF and SBF showed the highest amount of phytohormone production. From the present study, it is concluded that potassium solubilizing bacterial isolates has the potential to produce significant amount of phytohormones like IAA, cytokinin and Gibberellic acid. These potassium solubilizing bacterial isolates can be applied as potential biofertilizers for the development of crop in sustainable agriculture to replace the use of synthetic fertilizers.

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