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FTIR ANALYSIS OF AUXIN PRODUCING BACTERIA FROM RHIZOSPHERIC SOIL OF ORCHID

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Abstract

Indole acetic acid is one of the key phytohormones which play a vital role directly in the enhancement of plant growth. Auxin-producing bacteria inhabit the rhizospheric soil of orchid and can bring benefits to the host plant. Screening of plant growth promoting rhizobacteria and its characterization will play a significant role in crop improvement. In this context, the present study aimed for the isolation and screening of auxin producing bacteria from the rhizospheric soil of orchid. The bacterial strain was identified as *Kocuria rosea* through various morphological and biochemical analysis and confirmed as auxin producing bacteria through salkowshi reagent and FTIR analysis. The maximum IAA production was found at 120 hours incubation when fed with 0.2 mg/ml L-tryptophan.

Key words: auxin producing bacteria, Salkowshi reagent, FTIR, crop productivity.

Introduction

Plant growth-promoting bacteria (PGPB) are associated with the variety of plant species and habitually occur in different environments. PGPB is a plant growth-promoting rhizobacteria that colonize the rhizosphere, the rhizo-plane (root surface), or the roots itself, *i.e*. within radicular tissues. Irrespective of the diverse environmental availability, free-living rhizobacteria and symbiotic bacteria utilizes several identical mechanisms in order to enhance plant development and management of phyto-pathogens (Navarro-Torre *et al*., 2016). PGPR are indigenous to soil and are able to competitively colonize plant roots. An effective root colonist is a fundamental trait for PGPR in order to survive in the rhizosphere and root surface and to establish and effectively support host plant growth (Kamilova *et al*., 2005). Plant growth promoting rhizobacteria (PGPR) shows an important role in the sustainable agriculture industry. The increasing demand for crop production with a significant reduction of synthetic chemical fertilizers and pesticides use is a big challenge nowadays. Towards sustainable agricultural crops must be developed in qualitative and quantitative aspects.

To fulfill, one possibility is to use soil microorganisms (bacteria, fungi, algae, etc.) which increases the nutrient uptake capacity and efficient water change (Armada *et al*., 2014). PGPR can be used to enhance plant health and promote plant growth rate without any environmental impacts (Calvo *et al*., 2014).

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Materials and Methods

Soil collection

The rhizospheric soil was collected in a sterilized polypropylene covers from the orchid root at Pearl Orchid Centre, Palakad (Dt), Kerala.

Isolation of bacteria from rhizospheric soil

The soil samples were serially diluted and were spread on plates containing Nutrient Agar (NA) medium. The NA medium comprises, peptone-5g, NaCl-3g, beef extract-3g, agar-15g. The plates were kept for incubation at 28±2°C for 24 hours. Bacterial colonies were sub cultured several times on NA plates till the appearance of pure cultures. The isolates were stored in refrigerator on NA media for further studies. Then the isolate was confirmed through morphological and biochemical characterization.

Screening the isolates for its Auxin production

Bacterial cell suspension adjusted to 10^{-6} to 10^{-7} CFU ml^{-1} was inoculated in autoclaved NA broth supplemented with 0.2% tryptophan. Inoculated flasks were incubated at 30°C. After incubation, bacterial cells were removed

Table 1: Morphological and Biochemical Identification of auxin producing bacteria.

TEST	*Kocuria rosea*
Colony color	Orange
Shape	Coccus
Motility	Negative
Gram staining	Gram positive
Indole	+
Methyl red	+
Voges –proskauer	–
Citrate	–
Catalase	+
Oxidase	+
+ = Positive; – = Negative	

from culture medium by centrifugation at 10,000 rpm for 20 minutes. After centrifugation, auxin was detected by taking 1 ml of cell free supernatant and 2 ml of Salkowski reagent. (50 ml of 35% perchloric acid and 1 ml 0.5 M $FeCl_3$ solution)

Quantification of auxin producing bacteria

After the incubation period of 30 minutes in dark condition, the color changes occur from normal to red indicates the presence of auxin in the bacteria, the amount of IAA content was determined by UV-Visible Spectrophotometer at 535 nm. Different concentration of culture filtrate was estimated by using synthetic auxin as standard.

Effect on incubation period of IAA producing bacteria

The NA broth was prepared and pure bacterial colonies were incubated in orbital shaker in the condition with 100 rpm. During the incubation period, bacterial culture was examined for the production of auxin in every 24 hours intervals. Presence of auxin in the bacterial culture filtrate was confirmed only after the addition of Salkowski reagent.

Effect of various L-Tryptophan concentrations on IAA producing bacteria

L-tryptophan is considered as a precursor for IAA production because its addition to medium increases IAA production. Different concentrations of L-tryptophan between 0.1, 0.2 and 0.5 mg/ml were selected to find out the effect on IAA production.

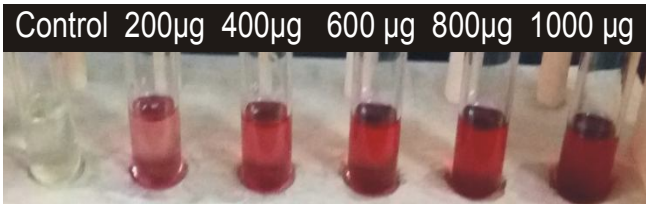

Fig. 2: Screening of auxin producing bacteria in different concentration of bacterial cultures.

Effect of shaking and static conditions on IAA producing bacteria

To determine the effect of static and shaking condition on IAA production, 2 sets of flasks with similar production media were inoculated. One set was kept in the environmental shaker at 100 rpm and the other set was maintained in static condition.

Extraction and purification of bacterial IAA for FTIR analysis

Single bacterial colony was inoculated in 200 ml of nutrient broth containing 0.2g/100ml of tryptophan and incubated at 30ºC for 96 hours on a shaker. Bacterial cells were separated from the supernatant by centrifugation at 10,000 rpm for 30 min. The supernatant was acidified to pH 2.5-3.0 with 1 N Hydrochloric acid and extracted twice with ethyl acetate. Extracted ethyl acetate fraction was evaporated in a rotator evaporator at 40ºC. The extract was dissolved in 3 ml of methanol and kept at -20ºC. The purified compound was identified as IAA by Fourier Transform Infrared [FTIR] spectrophotometer.

Results and Discussion

The bacterial isolate was screened using Bergey's manual of determinative of bacteriology MacFaddin, 2000) and identified as *Kocuria rosea* on the basis of its morphological and biochemical assay (Table 1). In addition, the auxin producing capability of *Kocuria rosea* was confirmed through Salkowski reagent test and the color changes reveals the presence of auxin (Fig. 2).

Effect of incubation period on growth and IAA production by *K. rosea*

Effect of incubation period on IAA production, maximum IAA production was observed at 120 hours due to the stationary phase growth of the bacteria. After 120 hours, the growth and IAA production become slow down due to the lag phase of bacteria. Similar reports are also recorded in various experiments of IAA production such as, 96 hrs in *Pseudomonas putida UB1* and *Sterptomyces sp*. VSMGT1014 (Bharucha *et al.*, 2013 and Harikrishann *et al.*, 2014). However variations

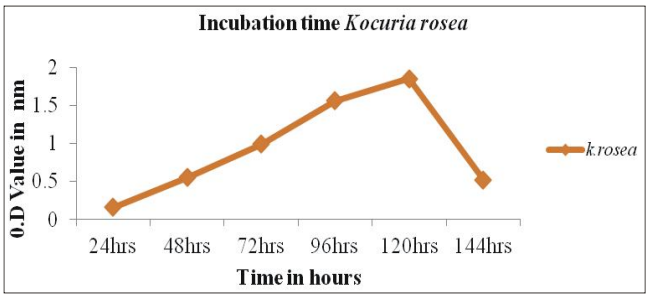

Fig. 3: Effect on incubation period of IAA producing bacteria.

in growth period (72 hrs) was recorded in same species with different strains of *Streptomyces* CMUH009 (Khamna *et al*., 2010).

Providing appropriate incubation period for the culture is another key factor for the enhancement of auxin production. Therefore, the qualitative and quantitative production of IAA by the bacterial isolate depends on its physicochemical conditions of the culture and environmental factors (Patil *et al*., 2011).

Effect of different Tryptophan with different concentration on IAA production by *K. rosea*

K. rosea shows the maximum production of IAA 57.75 μg/ml^{-1} while growing in the medium along with the tryptophan (2mg/ml) at 120 hours of incubation period. However, the IAA production was unfavorable when increasing the concentration of tryptophan from 2 mg to 5 mg/ml. Hence, changing the concentration of tryptophan in the culture media shows significant variations in IAA production (Lee *et al*., 2013).

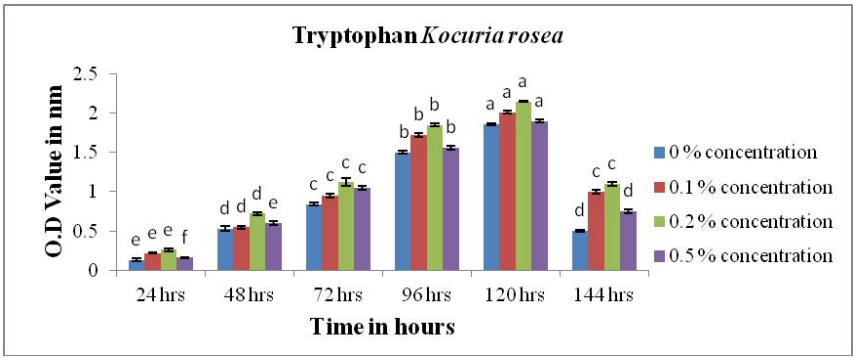

Fig. 4: Effect on tryptophan concentrations of IAA producing bacteria.

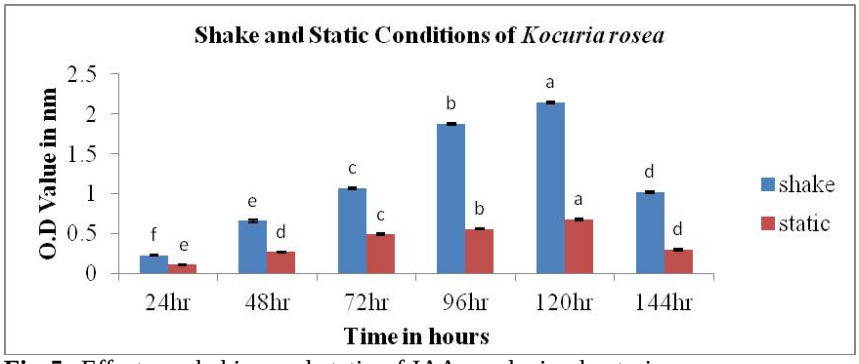

Fig. 5: Effect on shaking and static of IAA producing bacteria.

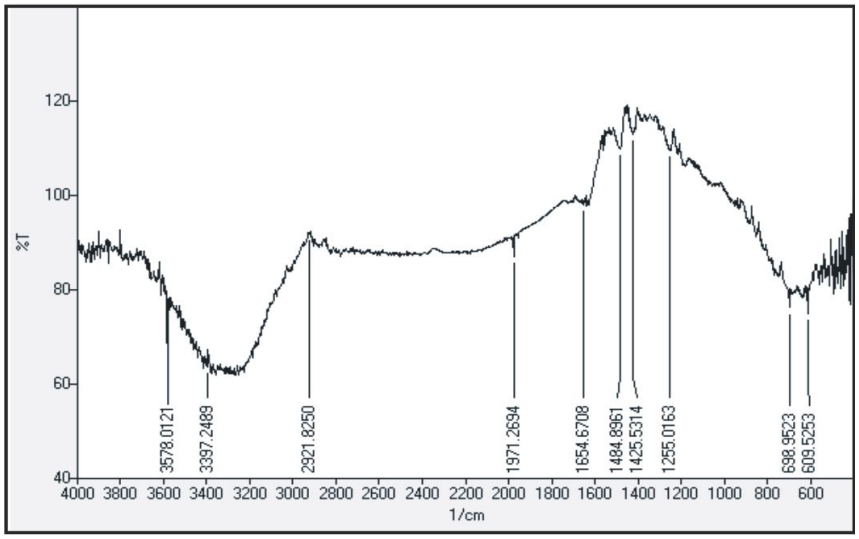

Fig. 6: FTIR analysis of *K. rosea*.

Effect of different Shake and Static conditions on IAA production by *K.rosea*

The maximum auxin production was recorded as 57.3 μg/ml^{-1} in the isolate kept under shaker for 120 hours at 100 rpm and the auxin production was very minimum and recorded as 3.5 μg/ml^{-1} in static conditions. The importance of continuous and even distribution of oxygen supply for the growth of culture and its auxin production was proven again (Bharucha *et al*., 2013).

Detection of IAA compounds in Fourier Transform-Infrared (FTIR) analysis of the isolate

The IR spectrum of the purified compound of *Kocuria rosea* showed NH frequency at 3397 cm^{-1} and a C=O frequency at 1654 cm^{-1}. The FTIR analysis of the functional group for the bacteria also confirmed that which was responsible for auxin. The characteristic (N-H) stretching of indole moiety is observed at 3339.22 cm^{-1} (N-H) bending and wagging was observed at 1642.32 cm^{-1} and 524.06 cm^{-1} Alkyl (-CH2) asymmetric stretching, symmetric stretching and bending was observed at 2979.51 cm^{-1} and 1453.11 cm^{-1}, respectively in *Pseudomonas stutzeri* strain (Patel and Patel 2014). IR spectrum of the purified compound showed OH frequency at 3389 cm^{-1} and a C=O frequency at 1698.4 cm^{-1} in the strain of *Klebsiella pneumonia* (Sachdev *et al*., 2009).

Conclusion

Increased the consumption of chemical fertilizers and pesticides has resulted in the accumulation of deposition of chemical compounds in the environment as well as the development of resistance in pathogenic microorganisms. To evade these

undesirable effects, it is of utmost importance to use biological agents, such as bio-fertilizers and bio-pesticides. The current investigation helps us to understand the association between the rhizospheric soil and the microbes for the provision of IAA. In conclusion, *Kocuria rosea* has activities of plant growth promotion through the secretion of several substances with particular reference to auxin (IAA). It can be a good candidate for a plant growth promoter used in agricultural production.

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