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Elucidating IAA producing *Kocuria flava* FA10 as a Potent PGPB and Biocontrol Agent

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Abstract

The use of chemical fertilizers and pesticides leads to environmental and public health problems. An alternative to these is the exploitation of beneficial microbes as biofertilizers and biocontrol agents. They are given foremost importance in agricultural sector for their impending role in sustainable crop production. Eco-friendly approaches inspire a wide range of application of plant growth-promoting bacteria (PGPB) for better nutrient uptake, plant growth, plant tolerance to abiotic and biotic stresses, and bio control property including disease management. One such bacterium, *Kocuria flava* FA10 was assessed for different PGP traits. Out of all PGP traits evaluated, it produced Indole-3-acetic acid (IAA) as high as 102µg/ml. It also possessed anti-fungal activity amongst different fungal phytopathogens making it a potent PGP bacterium as well as a biocontrol agent.

Keywords: *Kocuria flava*, Plant growth promoting bacteria (PGPB), Indole-3-acetic acid (IAA), Anti-fungal activity.

INTRODUCTION

The genus *Kocuria* was proposed by Stackebrandt [1], which was earlier classified in the genus *Micrococcus.* The members of this genus are gram-positive, coccoid, aerobic, non-encapsulated, and non-endospore forming belonging to the order of *Actinomycetales.* This genus of Actinobacteria has been isolated from different sources such as air, fermented sea food, mammalian skin, soil, the rhizoplane, freshwater, seawater, marine sediment, and desert soil [2]. *K. varians, K. rhizophila*, *K. flava*, and other unnamed species of *Kocuria* have been reported to produce large amount of indole-3-acetic acid (IAA) [3, 4]. Commonly known traits of PGPB include phytohormone production such as IAA, production of ammonia from nitrogenous organic matter, solubilization of phosphate and so on [5-7]. Despite of well-

documented history for species belonging to *Kocuria* genus as IAA producers, they have been poorly investigated for their potential as PGPB and biocontrol agents. In the present study, *Kocuria flava* FA10 was evaluated for different PGP traits namely IAA production, Phosphate solubilization Hydrogen cyanide (HCN) production, Ammonia production, Catalase activity, Amylase activity, Cellulase activity, Urease activity and Protease activity. Moreover, antagonism to fungal phytopathogens by *K. flava* FA10 was tested, which is an essential trait to categorize an organism as a biocontrol agent.

MATERIALS AND METHODS

Assessment of PGP traits

Production of Indole acetic acid (IAA) in the bacterial isolates was estimated by inoculation of Exponentially grown suspension of bacterial strain of FA10 in Nutrient broth (Himedia) containing 50 µg ml^{-1} L-tryptophan. The bacterial culture was kept at 28±2°C for 48 h and then centrifuged at 10,000 g for 10 mins. IAA concentration in the cell free culture supernatant was estimated using Salkowski reagent [8]. The system comprising of cell free supernatant and Salkowski's reagent (1:2 v/v) was prepared and incubated until red colors develops. Absorbance was measured at 535 nm. Indole acetic acid production was determined by using Indole acetic acid 100µg/ml as the standard.

A qualitative assay for Phosphate solubilization was performed in Pikovskaya medium containing tricalcium phosphate [9]. Bacterial culture was spot inoculated on agar plate and incubated at 30°C for 72 h. The presence of a clear halo zone around the culture spot indicates the P solubilization capacity of the isolate. Hydrogen cyanide (HCN) production was evaluated by the qualitative method [10] by inoculating the bacterial strain FA10 on Nutrient agar medium amended with 0.44% glycine. Circular Whattman filter paper no.1 soaked in picric acid (0.05% solution in 2% sodium carbonate) was placed in the lid of each Petri plate. A color change of the disc from yellow to reddish-brown was considered as an indication of HCN production.

Detection of ammonia production was done by adding 1 ml Nessler's reagent to 1 ml of 72 h old cell free supernatant of the bacterial strain FA10 grown in peptone broth and making the final system volume to 10 ml. Development of yellowish brown color at 450 nm gave a positive test [11]. Ammonia production was determined by using ammonia solution 1 µmol ml^{-1} as the standard. Presence of catalase was checked qualitatively by the method described by [12]. Where, 6% H_2O_2 was added on the colonies grown on Nutrient agar plates after incubation of 48 h. Effervescences of O_2 released from the bacterial colonies upon addition of H_2O_2 indicated the presence of catalase activity.

Qualitative estimation of amylase production was performed on starch agar plate by starch hydrolysis test [13]. Bacterial strain FA10 was streaked on starch agar plates with starch as the only carbon source. After incubation at 30±2ºC for 48h, plates were

flooded with Gram's iodine (Gram's iodine- 250 mg iodine crystals added to 2.5 gm potassium iodide solution and 125ml of water). A deep blue colored starch-iodine complex is produced upon addition of iodine solution. The amylase producers would display zone of clearance and no blue color would be formed surrounding the zone. Qualitative estimation of Cellulase was done by streaking bacterial strain FA10 on basal medium agar containing Carboxymethyl cellulose (CMC) (0.5% w/v) as the sole carbon source. After incubation at 30±2ºC for 48h, all the plates were stained with 1% (w/v) Congo-red solution for 15 min and discolored with 1 M NaCl for 15 min[14]. The zone of degradation around bacterial colonies indicate positive for this test.

For qualitative estimation of urease production, bacterial strain FA10 was grown on urea agar base (Himedia) supplemented with 5 ml of 40 % urea solution in 100 ml medium. A change of color from yellow to pink indicated urea hydrolysis by the bacteria [15]. As well, Protease activity was determined by spot inoculating culture on M9 (1%w/v) agar plates supplemented with Casein (0.5%). Plates were incubated at 30ºC for 48 h, clear zone around the colony showed protease activity of the bacterial isolate.

Agar well diffusion method described by [6] was used to assess antifungal activity. Agar plates containing nutrient agar and PDA (1:1) was used onto which, heavy inoculum of spores (2×10^8 cells ml^{-1}) were spread to get carpet growth and wells of 8 mm diameter were punched into the agar medium which were filled with 100µl of bacterial culture. The plates were incubated for 120 h at 27 ± 2ºC. Antifungal activity was observed in terms of inhibition index by clear zone produced as a result of fungal growth inhibition. Test fungus used were *Aspergillus niger* and *Trichothecium roseum*

RESULTS AND DISCUSSION

Assessment of PGP traits

Among nine different traits assessed, *K. flava* FA10 tested positive for IAA production, Ammonia production, Catalase and protease activity (Table: 1).

Table 1. Different PGP traits exhibited by *K. flav*a FA10

PGP traits	Result
Indole acetic acid production	**+ (102µg/ ml)**
Phosphate solubilization	-
Hydrogen cyanide (HCN) production	-
Ammonia production	**+ (36µg/ ml)**
Catalase activity	**+**
Amylase activity	-
Cellulase activity	-
Urease activity	-
Protease activity	**+**

IAA production was highest amongst the rest producing amounts as high as 102µg/ml (Fig: 1). Ammonia produced was 36µg/ml (Fig: 2). *K. flava* FA10 was positive for catalase. Strong effervescences of O_2 evolved when 6 % H_2O_2 solution was flooded on the colonies grown on Nutrient agar indicating positive qualitative result for catalase production. Protease activity too gave positive result. IAA production results in increased root, shoot length, and even faster seed germination rates. Whereas, Ammonia helps in plant growth, proteases protects against the plant pathogens by degrading its cell wall and catalase enhance defense mechanism in plants against reactive oxygen species (ROS). Hence, resulting in a disease free and healthier crop on application of *K. flava* FA10 on it.

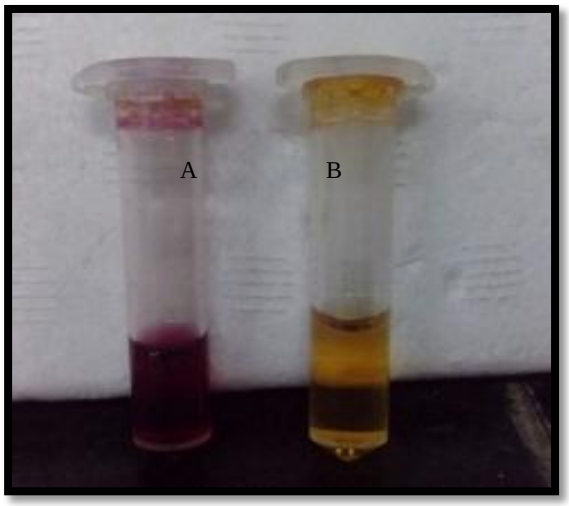

Figure 1. IAA production by *K.flava* FA10 (A), compared to control (B).

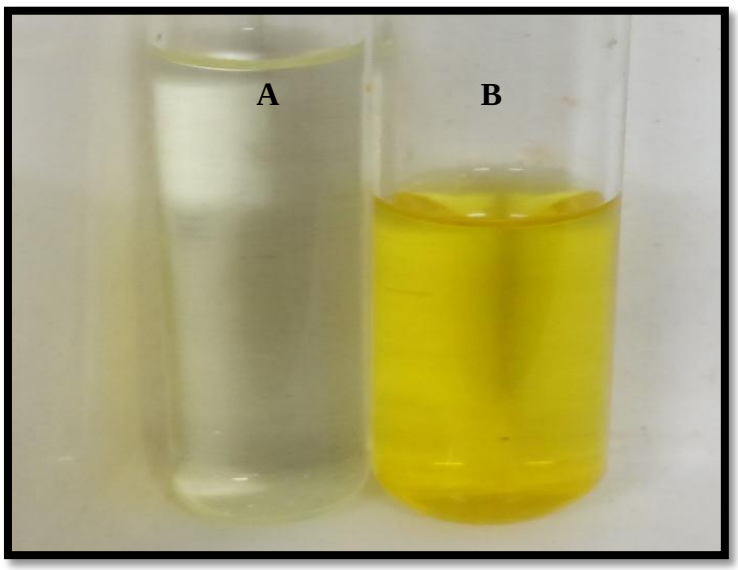

Figure 2. Ammonia production by *K.flava* FA10 (B) as compared to control (A).

Assessment of Anti-fungal activity

Antifungal activity attributed by *K. flava* FA10 was tested against *A. niger* and *T. roseum*. Figure 3 shows the growth inhibition of *A. niger* and *T. roseum* by *K. flava* FA10. Thus, it is apparent that *K. flava* FA10 suppresses the growth of these fungi suggestive of its antifungal activity. Suppression on such fungal phytopathogens is due its cell wall lysis by the bacterial antagonists. This property is attributed to bacterial antagonists due to secretion of certain lytic enzymes such as Keratinase and Chitinase.

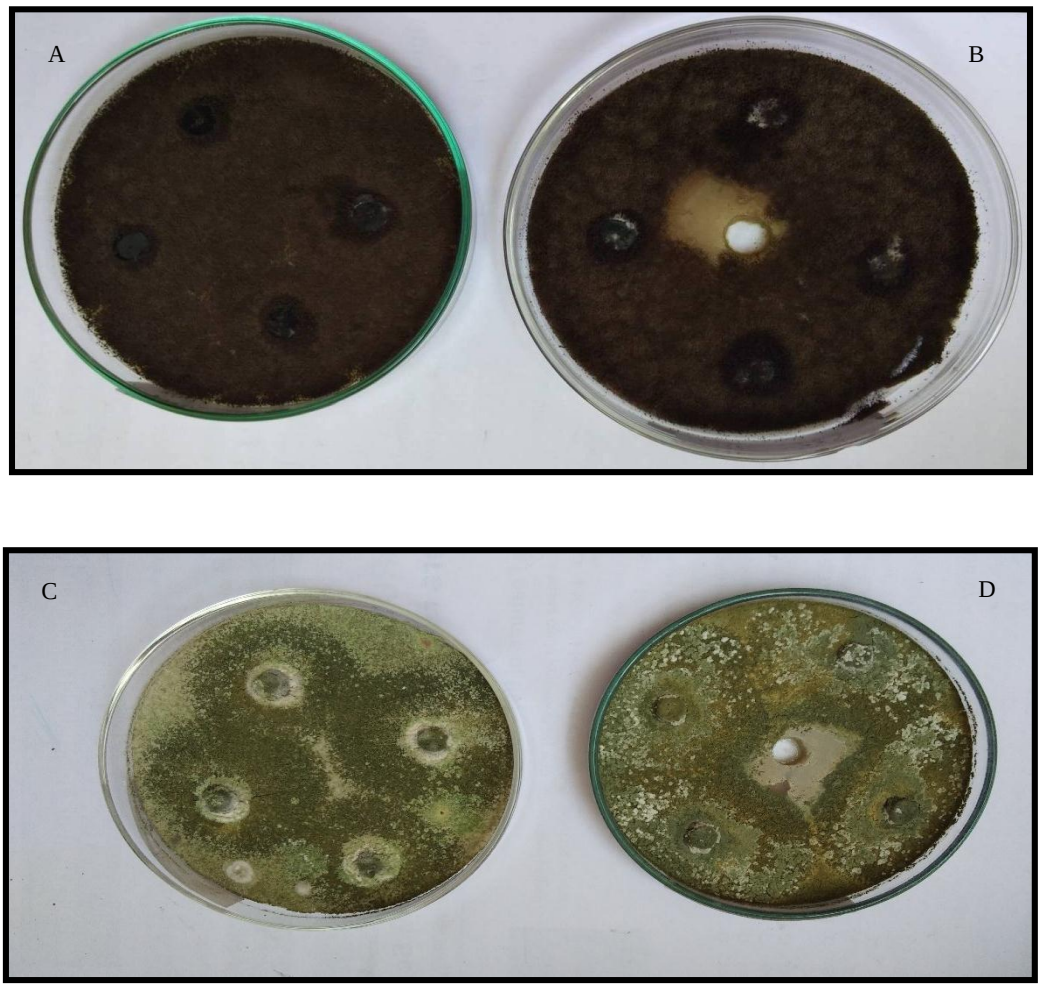

Figure 3 The inhibition zone of *A. niger* by *K. flava* FA10 (B), as compared to uninoculated control (A). Whereas, (D) demonstrates inhibition zone of *T. roseum* as compared to uninoculated control (C).

CONCLUSION

From the above result analyzed in this study, *K. flava* FA10 has good amount of PGP traits as well it has been tested positive for anti-fungal activity too. Hence, *K. flava* FA10 may be further exploited as a potent PGPB as well as a bio control agent in

managing fungal phytopathogens. So if *K. flava* FA10 is to be applied to crops, it will not only protect them against fungal phytopathogens, will result in healthier plant too.

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