



GROWTH PROMOTING CHARACTERISTICS OF ENDOPHYTIC BACTERIA ISOLATED FROM *COSTUS IGNEUS* PLANT

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Abstract

Medicinal plant-endophytic bacteria interactions modulated production of secondary metabolites finds wide range of application in agriculture in growth promotion and stress tolerance, medicines and industries. Endophytic bacteria were isolated from *Costus igneus* (insulin plant; (IP) from roots and leaves (L) explants, using King's B (K) and NMS (N) media. Based on the biochemical characteristics determined by Biolog GEN III and its extensive species library isolates were identified as *Terrimonas ferruginea* (IPNR), *Massilia lutea* (IPKR), *Klebsiella variicola* (IPNL) and *Raoultella terrigena* (IPKL). All the four isolates showed the production of indole acetic acid (IAA) which was high in root endophytic isolates. The siderophore production was strain specific characteristics. The strains were positive to catalase activity. All the endophytic bacteria isolate solubilized tricalcium phosphate and chitin. Solubilization of insoluble potash (mica) and zinc (ZnO₂) and was more in root isolates. The isolates were sensitive to streptomycin, kanamycin, tetracycline and resistant to ampicillin and nalidixic acid. Among the isolates IPKR exhibited maximum production of IAA and siderophore and P, K, Zn and chitin solubilization.

Key words: *Costus igneus*, root and leaf endophytic bacteria, Biolog Gen III Identification, Growth promoting characteristics.

Introduction

Bacteria colonizing internal plant tissues, endophyte bacteria establish symbiotic or mutualistic association with almost all parts of plant species on the earth. They probably contribute to the evolutionary fitness of host plant by producing of secondary metabolites. Endophytic bacteria produce bioactive compounds by which they help to provide resistance against diseases and survival to stresses (Strobel *et al.*, 2004). Bacterial bacteria endophytes affect the metabolic potentials may contribute to growth and stress tolerance of plants. Endophytic mitigated production of secondary metabolite is wide spread phenomenon by aromatic and medicinal plants. Some metabolites are produced by plants and associated bacteria interactions. Endophytic bacteria secrete a wide range of bioactive secondary metabolites such as alkaloids, benzopyranones, chinones, flavonoids, phenolics, quinones, steroids, terpenoids, tetralones, xanthenes and others (Tan

and Zou; 2012. Singh *et al.*, 2017) exhibiting a wide-range of applications in agriculture, medicines and industries (Gunatilaka, 2006).

A large number of endophytic bacteria produce indole acetic acid (IAA). *Klebsiella* sp. Sal 1 and *Enterobacter* sp. Sal 3, strains showed IAA-degrading ability while *Herbaspirillum* sp. Sal 6 has been reported as a potent IAA producer (Dhungana *et al.*, 2019). Indole acetic acid production is a major property of some rhizospheric bacteria that promote the plant growth. The rhizospheric bacteria *Azotobacter*, *Pseudomonas* and *Spirillum* of the marigold plant exhibited IAA production with positive test for, catalase, oxidase, urease and starch hydrolysis. The marigold seeds treated with bacterial strains showed more root and shoot growth in comparison to control (Maharana, P.K. 2019).

Siderophores, an iron chelating secondary metabolite produced by different endophytic bacteria assist the plant growth by providing iron (Maheshwari *et al.*, 2019).

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Although, iron is abundant in soil, but its availability is limited due to less solubility of the Fe^{3+} ions. The divalent (Fe^{2+}) state is easily oxidized to the trivalent (Fe^{3+}) which is precipitated in soil in the form of oxide or hydroxide. Several endophytic bacteria have the capacity to produce this low molecular weight iron chelating compound siderophore in different form such as Hydroxymate, Catecholates etc. This iron chelating agent can make insoluble form of iron into soluble form by mineralization and sequestration for the growth and development of plant (Pahari *et al.*, 2017).

Catalase, an enzyme catalyzes the decomposition of hydrogen peroxide to water and oxygen and thus protects the cells from oxidative damage by reactive oxygen species (ROS). It's presence in the bacterial cells is largely governed by the growth conditions and the medium composition. (<https://en.wikipedia.org/wiki/Catalase>).

The solubilization of inorganic phosphate and enzymatic mineralization of organic phosphates is another growth promoting trait exhibited by endophytic bacteria. *Aneurinibacillus* sp. and *Lysinibacillus* sp., endophyte of banana tree, effectively solubilized tricalcium phosphate and soy lecithin which reduced the pH of liquid medium and showed acid phosphatase activity (Matos *et al.*, 2017). Potassium (K) is required as an essential macro element in agricultural production systems, since the deficiency of K usually reduced crop yield. Potassium-solubilizing bacteria (KSB) in forest and rubber tree plantation rhizospheric soils in Myanmar showed the microbial abundance of KSB in the plantation soil higher than that of the forest soil. KSB accounted for less than 5.47% of the total bacteria detected in the soil samples, indicated the increasing use of KSB for restoring soil and reducing the use of chemical fertilizers (Dong *et al.*, 2019). Zinc solubilizing bacteria in rhizospheric region solubilize zinc oxide (ZnO). Inoculation of zinc solubilizing isolates, *Bacillus* sp. (ZM20), *Bacillus aryabhatai* (ZM31 and S10) and *Bacillus subtilis* (ZM63) increased the growth of maize plants (Mumtaz *et al.*, 2017). The endophytic bacterial isolates from soybean and summer mung bean rhizosphere have ability to solubilize zinc oxide (ZnO) and zinc phosphate $Zn_3(PO_4)_2$ (Sharma *et al.*, 2014).

Endophytic bacterial secondary metabolites have antimicrobial properties (Indrawati *et al.*, 2018). They act as plant crops' biotic and abiotic stressors by stimulating immune responses, aggressive colonization and excluding plant pathogens by niche competition. They exhibit antioxidant activities and phenylpropanoid metabolism, which indirectly stimulates plant defense,

structural support, and survival strategy molecules. Many endophytic Actinobacteria produce metabolites with antimicrobial and antitumor activities useful in agriculture, veterinary, medicine, environment and industries. The high endophytes diversity and their adaptation to various abiotic and biotic stresses make them a suitable and unlimited source of novel metabolites, whose applications could reduce the use of agrochemicals in food production. (Ek-Ramos *et al.*, 2019).

Costus igneus commonly called as Insulin plant has antidiabetic sugar lowering property (Sabu, 2006). Native of south and central America, the *Costus igneus* plant was introduced in south India during 2002-03 where it is cultivated as an ornamental plant (Merina, 2004). The different plant parts of the *Costus* species are used for scabies and stomach ailments, high fever, blisters, burns and against snake bite (Rathore and Khanna, 1978; Gruenwald *et al.*, 2000 and Warriar *et al.*, 1994). The extract of rhizome is used to make sexual hormones, contraceptives and also used for treating burning sensation, constipation, leprosy, asthma, bronchitis, anaemia and other skin ailments (Bown, 2008).

Keeping in view the information above, the growth promoting traits of some endophytic bacterial isolated from roots and leaves of *Costus igneus* were investigated. Further, the biochemical characteristics of the isolates determined by Biolog were used for identification of the strains.

Materials and Methods

Collection of root and leaf from pea plant:

The healthy roots and leaves were collected from insulin plant (*Costus igneus*), grown in the soil and pots in the Botanical Garden of Department of Botany, Institute of Science, Banaras Hindu University, Varanasi, Uttar Pradesh, India. The plants were six-month-old and of about 50 cm in height.

Freshly collected root and leaves from insulin plant were cleaned thoroughly with tap water. Five roots and leaves were cut into 1 cm pieces and transferred to sterile 250 ml Erlenmeyer flask containing 50 ml of sterile water. The flasks containing explants were shaken for 30 minutes on a shaker and washed several times with sterile distilled water. The explants were then aseptically transferred to another sterile flask and surface sterilized as follows: the root and leaf explants were placed in 95% ethanol for 1 min followed by washing with sterile distilled water. In the second step, explants were surfaces sterilized with 0.1% $HgCl_2$ for 5 min, and washed several times with sterile distilled water (Chaintreuil *et al.*, 2000). Root and

leaf explants were sliced (0.5 mm) with sterilized razor and six sliced root and leaf explants were placed aseptically on King's B medium (King's *et al.*, 1954) and NMS medium (Whittenbury, *et al.*, 1970)) agar plates and incubated at 30°C for 3-7 days. Well-developed single colonies growing around root and leaf explants were picked up and transferred repeatedly on agar plates to observe consistency in colony morphology and growth. Finally, clones based on their colony characteristics and growth on their respective media of isolation were selected and stored on the slants in Bakelite tube of same media at 5°C. Four endophytic isolates IPNR, IPKR, IPNL and IPKL (where I = insulin, P = plant, N = NMS medium, K = King's medium, R = root and L = leaf) were selected for further study.

Biochemical characteristics and identification of endophytic by Biology

Freshly grown bacterial colonies grown on nutrient agar were picked up and suspended in of 0.85% 5 ml saline solution. Bacterial suspensions were adjusted in IF-A to 90-98% transmittance (T90) using a Biolog turbidimeter. The suspensions (150µL) was dispensed into each well of a Biolog GEN III microplate. The plates were incubated at 30°C in an Omnilog incubator. After 24 and 48 hr of incubation, the phenotypic fingerprint of purple coloured well were compared to the Biolog extensive species library.

Catalase assay

Catalase test was performed by adding 2-3 drops of 3% H₂O₂ with single bacterial colony on clean grease free glass slide and mixed with inoculation loop. Immediate formation of gas bubble showed the positive test for catalase enzyme.

Indole acetic acid (IAA) production

IAA production by the bacterial strains was estimated in the cells grown in nutrient broth supplemented with 100 µg/ml filter sterilized L- tryptophan. The cultures were incubated at 30°C for 48 hours with continuous shaking on a gravetory shaker. Cell biomass was centrifuged at 10,000g for 15 min. at 4°C and IAA produced was estimated by adding 4 ml of Salkowasky reagent (1 ml 0.5 M FeCl₃ in 50 ml of 35% perchloric acid) in the 2 ml culture supernatant. After thorough mixing, the absorbance was measured at 530 nm after 30 min (Gordon and Weber, 1951). The amount of IAA was determined by using the standard curve (10-100µg/ml IAA) and calculated by following equation,

$$y = mx + c \text{ (in } \mu\text{g/ml)}.$$

Where

y = O.D. of bacteria culture.

m = O.D. of blank solution

x = amount of IAA produces by bacteria isolates

c = Zero (constant)

Siderophore production

The endophytic bacterial strains were tested for the siderophore production on Chrome azurol sulphionate (CAS) containing nutrient agar medium (Schwyn and Neilands, 1987). The plates spotted with 10µl bacterial suspension (10⁸/ml) and was incubated at 28°C for 3-4 days in a BOD incubator. The deep yellow to orange colour developed surrounding the colony was a positive indication for siderophore production.

Phosphate solubilization

Endophytic bacterial isolates were examined for their phosphate solubilizing activity on Pikovskaya nutrient agar medium. (Pikovskaya, 1948). The petridishes were spotted with 10µl of exponentially grown culture. The inoculated plates were kept at 28°C for 7 days (Yanni *et al.*, 2001). The diameter of the solubilized area including bacterial growth was measured and phosphate solubilization activity was expressed as mm radial area solubilized/h.

Potassium solubilization

K- solubilization assay was performed on selective agar medium containing 1 g potash (mica) powder (9.60% K₂O). The plates spotted with 10µl of exponentially grown cultures of endophyte bacterial isolates were incubated under growth conditions (Yanni *et al.*, 2001). The radial area of the K-solubilized zone including colony was measured. The solubilization activity was expressed as mm radius solubilized/h.

Zinc solubilization

Zinc solubilization by endophytic isolates was assayed as described by Saravanan *et al.*, (2016). The clearing zone around colony growing in presence of zinc carbonate and zinc oxide was recorded and solubilization activity was expressed as mm radius solubilized/h.

Chitin solubilization

Chitin solubilizing activity of the endophytic isolates was determined in chitin containing nutrient agar plates. The plates spotted with 10µl of exponentially grown cells were incubated under growth conditions (Yanni *et al.*, 2001). The radius of solubilized clear zone area including bacterial colony growth was detected and the activity was expressed as mm radial area solubilized/h.

Antibiotic sensitivity

Antibiotics sensitivity of endophytic bacterial isolates

was determined on nutrient agar plates containing filter sterilized (0.22 mm Millipore membrane) discs of Kanamycin, Ampicillin, Chloramphenicol, Ciprofloxacin, Streptomycin, Tetracycline, Neomycin, Rifampicin and Nalidixic acid. Each agar plate was divided into six equal sectors and spot inoculated with 10 µl of exponentially grown bacterial culture. The plates were incubated at 30°C for 7 days (Maatallah *et al.*, 2002).

Results

Biochemical characterization (Biolog GEN III) for identification of bacterial isolates:

The endophytic bacterial isolates IPNR, IPKR, IPNL, IPKL (where I stand for insulin, P for plant, N for NMS medium, K for King’s B medium, R for root and L for leaf) were inoculated in Biolog Gen III microtiter plate to observe various carbon and nitrogen sources utilization

pattern, growth at different pH and salt (NaCl) concentrations and tolerance to some toxicant. The data obtained were used for identification using Biolog extensive species library. On the basis of comparison to library data the endophytic bacterial isolated were identified as follows as *Terrimonas ferruginea* (IPNR), *Massilia lutea* (IPKR), *Klebsiella variicola* (IPNL) and *Raoultella terrigena* (IPKL). Fig. 1.

Production of IAA by endophytic bacterial isolates

All the four endophytic bacterial isolates produced an appreciable amount of IAA in nutrient broth supplemented with 100 µg ml⁻¹tryptophan. The highest amount of IAA was produced by isolate IPNR (21.2µg ml⁻¹), followed by IPKR, IPNL and least by IPKL. There was a gradual increase in the IAA production with the time of incubation and after 48 hour the maximum IAA production was by IPNR (47.3µg ml⁻¹), followed by IPKR

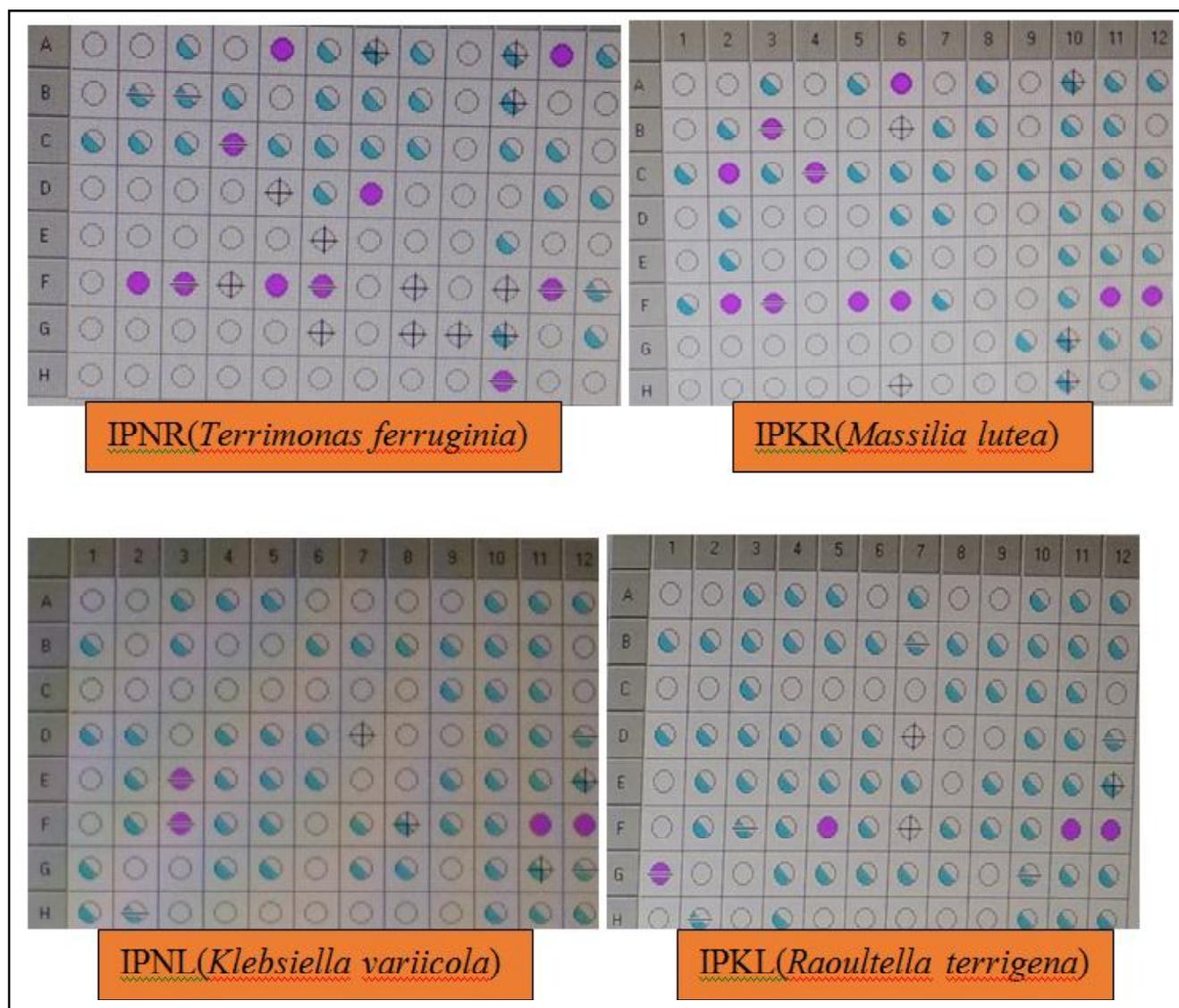


Fig. 1: Biolog based identification of bacterial isolates on Gen III plates.

(42.1 $\mu\text{g ml}^{-1}$), IPNL (33.2 $\mu\text{g ml}^{-1}$) and isolate IPKL IAA(29.8 $\mu\text{g ml}^{-1}$) The results undiated that root endophytic bacteria were good IAA producers than the leaf isolates. Fig. 2.

Siderophore production

Out of four endophytic bacterial isolates, the siderophore production was significant in only two isolates. The isolates IPNR and IPNL were positive for carboxylate types of siderophore while the other two isolates IPKR and IPKL did not produce any observable amount of siderophore. Fig. 3.

Catalase activity

All the endophytes bacterial isolates IPNR, IPNL, IPKR and IPKL were positive for catalase test Based on the intensity of bubble production the bacterial isolate IPKR and IPKL exhibited high catalase activity while enzymatic activity in IPNL and IPNR isolates was low Fig. 4.

Phosphate solubilization

The potential of endophytic bacterial isolates of *Castus igneus* for insoluble tricalcium phosphate solubilization activity was tested *in-vitro* on Pikovskaya agar medium. Two root endophytic isolates IPNR and IPKR showed the greater ability to solubilize tricalcium phosphate. The maximum solubilization activity was exhibited by root endophyte IPNR (0.56 mm hr^{-1}) and IPKR (0.26 mm hr^{-1}) followed by leaf isolates IPKL (0.22

mm hr^{-1}) and IPNL (0.20 mm hr^{-1}), leaf isolates. The results indicate that root endophytes were efficient phosphate solubilizers as compared to leaf endophytic bacteria of insulin plant. Fig. 5(A) and Fig. 6(A).

K-Solubilization by endophytic bacterial isolates

The endophytic bacterial isolates of insulin plant were tested *in-vitro* for potash solubilization (mmh^{-1}) activity

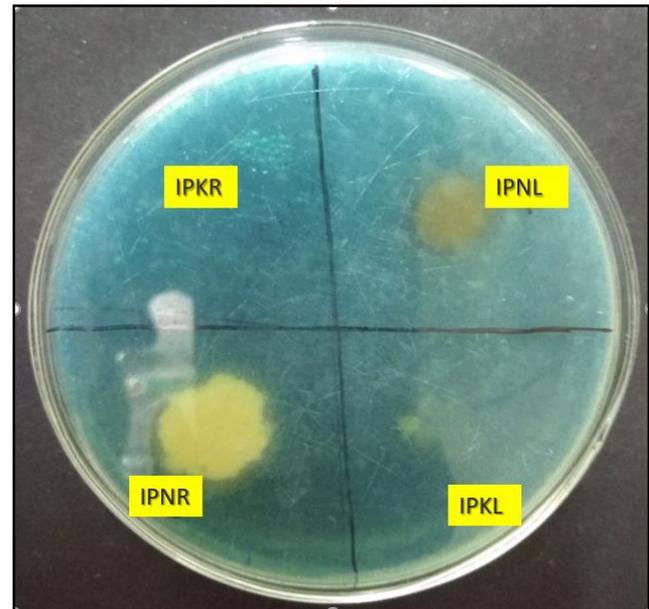


Fig. 3: Siderophore production by endophytic bacterial isolates.

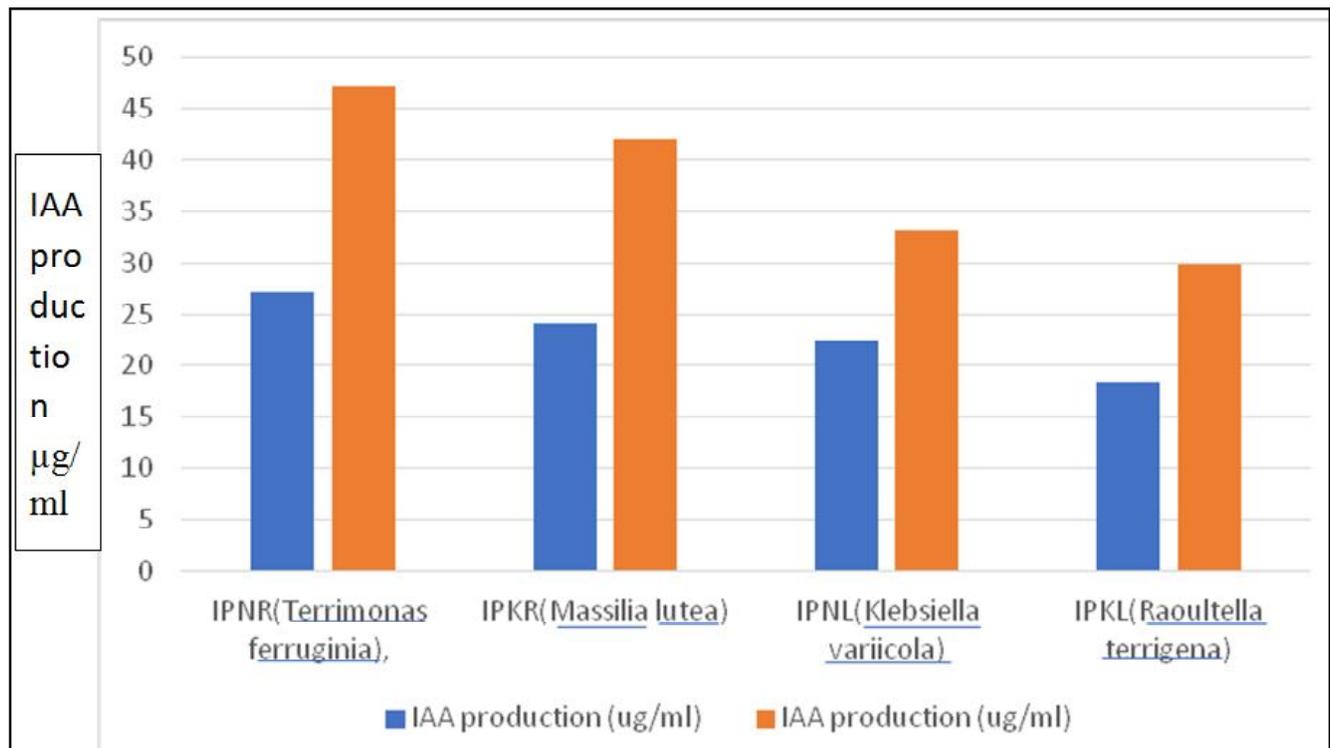


Fig. 2: IAA production by Bacterial Isolates at 24 h (blue color) and 48 h (white radish).

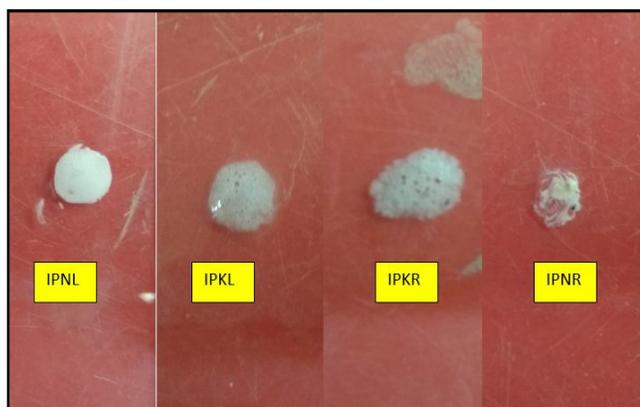


Fig. 4: Catalase activity (bubble formation with H₂O₂) of endophytic bacterial isolates.

on Aleksandrov’s nutrient agar medium supplemented with of mica powered felspar (1 gl⁻¹) an insoluble form of K. The maximum potash solubilization activity was observed by IPNR (0.20 mmh⁻¹) followed by IPKR (0.17 mmh⁻¹) and IPNL (0.13 mmh⁻¹) The leaf endophytic isolate IPKL did not solubilized potash Fig. 5(B) and Fig.6 (B).

Zinc solubilization property

An experiment was conducted to screen Zn

solubilizing in endophytic bacterial strains of insulin plant. Zn solubilization activity determined in zinc oxide (ZnO) and zinc carbonate (ZnCO₃) supplemented nutrient agar media the results have been presented in Fig 2. Only two root endophytic isolates (IPNR and IPKR) showed zinc solubilization which was high in IPNR (0.32 mm/hr) than IPKR (0.11 mm/hr). Two endophytic leaf isolates IPKL and IPNL did not show appreciable amount of zinc solubilization activity Fig. 5(C) and Fig. 6(C).

Chitin Solubilization property

Chitin are biomolecule and some bacteria can degrade chitin by secretion of chitinase enzyme. The endophytic bacterial isolates IPNR, IPKR, IPKL and IPNL were tested *in-vitro* for chitin solubilization activity on chitin supplemented nutrient agar media. All the four endophytic isolates showed the ability to degrade chitin. The maximum activity was in IPNR followed by IPKL, IPNL and IPKL in decreasing order Fig.5(D) and Fig. 6(D).

Antibiotic sensitivity of endophytic bacterial isolates

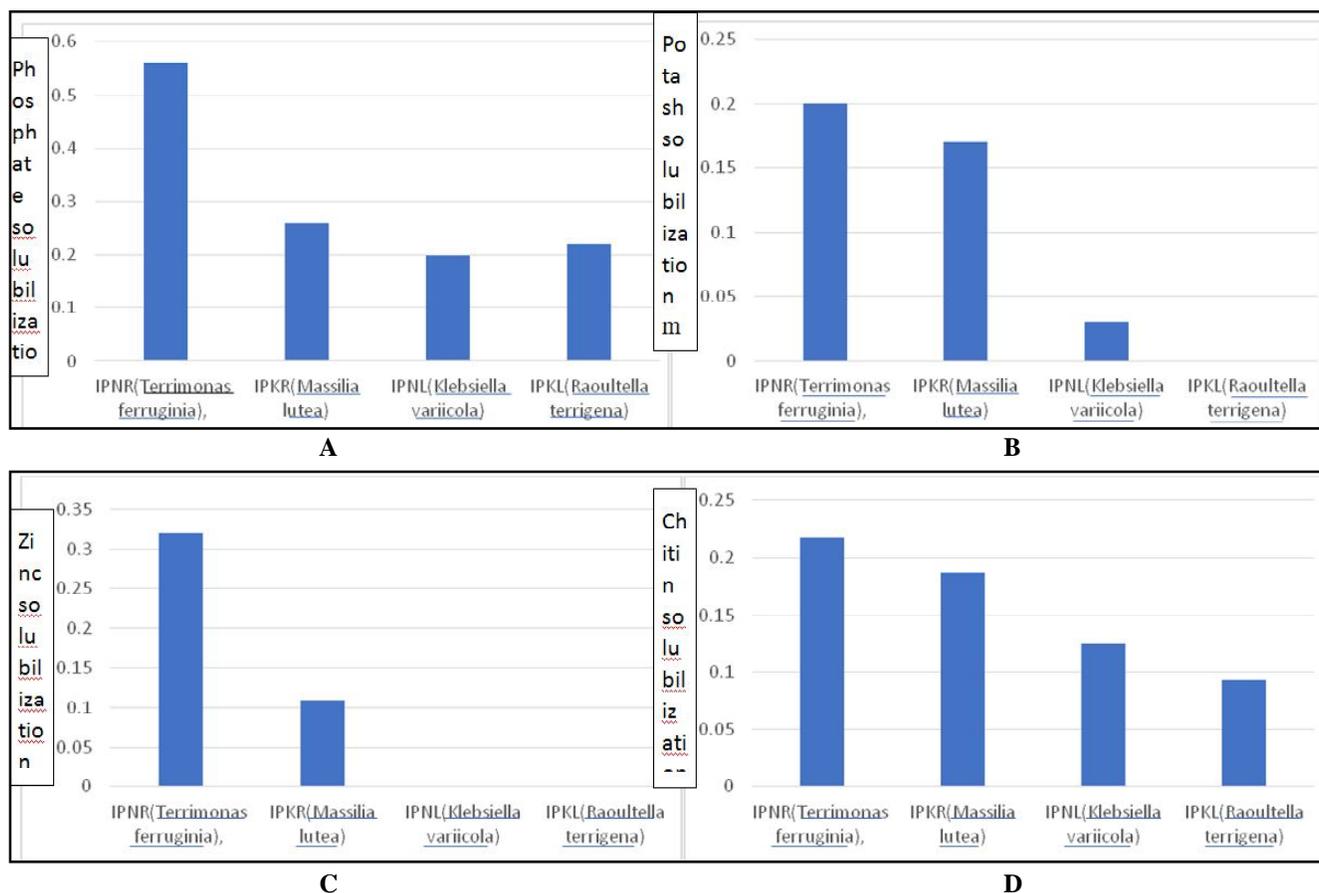


Fig. 5: Phosphate (A), Potash (B), Zinc(C) and Chitin(D) solubilization(mmh⁻¹) by four endophytic bacteria of *Castus ignuos* plant.

Antibiotic sensitivity of endophytic bacterial isolates IPNR, IPKR, IPKL and IPNL was observed on Ciprofloxacin (Cip), Erythromycin (Ery), Streptomycin (Stm), Nalidixic acid (Nda), Ampicillin (Amp), Kanamycin (Knm), Tetracycline (Tet), Rifampicin (Rmp) and Neomycin (Neo) containing disc on the lawn of endophytic bacteria on nutrient agar plates and incubated at 30°C for 5 days. All endophytic bacterial isolates showed resistance to Nalidixic acid and Ampicillin. Isolates IPNR, IPKR and IPNL were resistant to

Erythromycin and only IPNR showed resistance against Neomycin antibiotic. These endophytic bacterial isolates were sensitivity to Ciprofloxacin, Streptomycin, Kanamycin, Tetracycline and Rifampicin antibiotics (Fig.7 Table 1).

Discussion

Fourty two (22 from root and 20 from leaves) clones were isolated from *Costus igneus* plant based on the growth behavior two isolates each from root and leaf

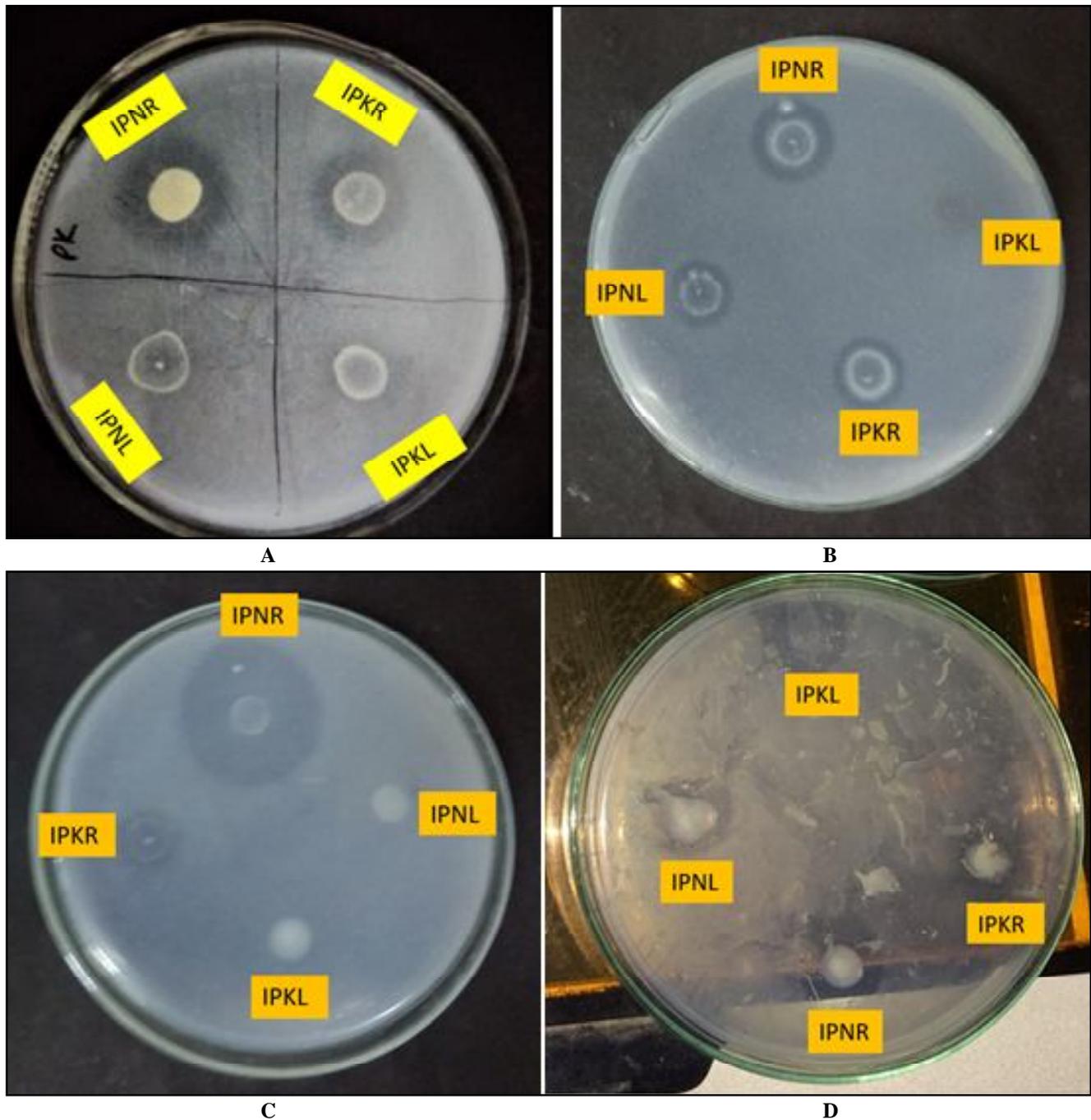


Fig. 6: phosphate (A), Potash(B), Zinc(C) and Chitin(D) solubilization by four endophytic bacterial isolates (clear zone indicates the solubilization areas).

were selected for further studies. The isolates were inoculated in Biolog Gen III microtiter plate to observe various carbon and nitrogen sources utilization pattern, growth at different pH and salt (NaCl) concentrations and tolerance to some toxicant for the identification of isolates using Biolog extensive species library system. Endophytic bacterial isolates were identified as *Terrimonas ferruginea* (IPNR), *Massilia lutea* (IPKR), *Klebsiella variicola* (IPNL) and *Raoultella terrigena* (IPKL).

IAA production is widespread among environmental bacteria that inhabit soils, waters, but also in plant and animal hosts. Distribution and substrate specificity of the involved enzymes for IAA production suggests that IAA

formation pathways play a role beyond plant-microbe interactions. (Patten *et al.*, 2013) *Enterobacter cloacae* can produce IAA, from aromatic and branched-chain amino acids. (Parsons *et al.*, 2015). All four endophytic isolates were capable to produce IAA in significant amount. Isolate IPNR a root endophyte produces maximum amount of IAA while the least amount was produce by IPKL (leaf endophyte). The results suggested that the root endophytic bacteria are more efficient producer of IAA probably due to availability of substrate inside the root tissues. The results of quantitative analysis of IAA production from 17 bacterial isolates showed that *Enterobacter ludwigii* FB Endo 135 produced the highest IAA, while the lowest amount was formed by Azm

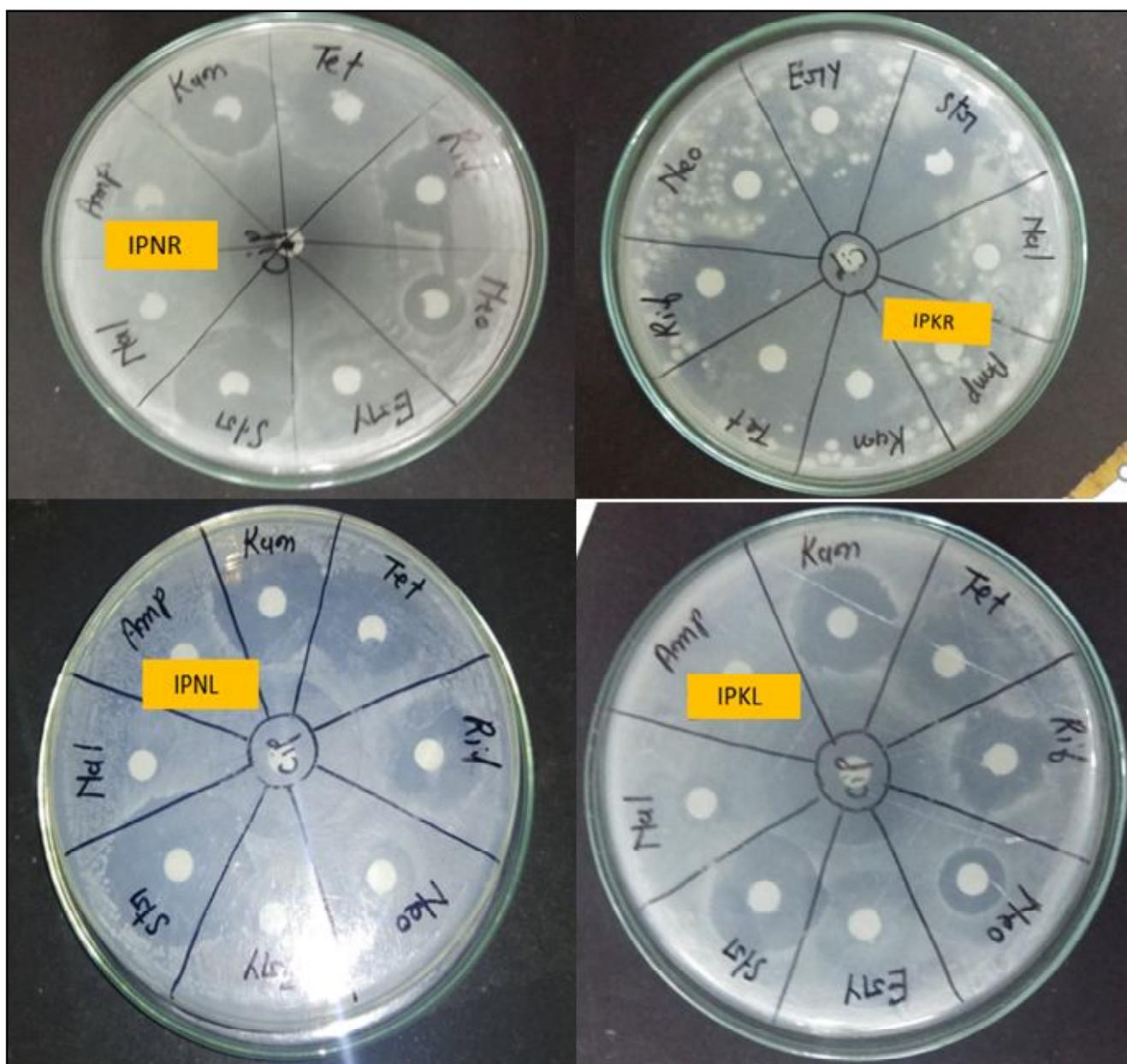


Fig. 7: Antibiotic of sensitive by endophytic bacterial determined by different antibiotic disc.

Table 1: Sensitivity of endophytic bacterial isolates to different antibiotics.

Endophytic isolates	Cip	Ery	Stm	Nad	Amp	Kamm	Tetr	Rif	Neo
IPNR	+++	*	++++	*	*	+++	+	++	*
IPKR	++++	*	++	*	*	+++	++++	+++	+
IPNL	*	*	++++	*	*	+++	+++	+++	+
IPKL	++++	++	++++	*	*	++++	++	+++	++

++++ (Highly sensitive), +++ (Moderately sensitive), ++ (Low sensitive), + (Resistance), * (Highly Resistance).

1.7.2.12. (Susilowati *et al.*, 2018). Isolates DM and K1K1 producing high IAA levels were used for bacterization of mungbeans enhanced the number of lateral root formation. (Herlina *et al.*, 2017).

Siderophores are iron-chelating compounds secreted by microorganisms such as bacteria and fungi. They help to transport iron across cell membranes. The endophytic bacteria produce different types of this low molecular weight compounds. In the present study endophytic bacterial isolate IPNR and IPNL showed maximum siderophore production which indicated that both root and leaf endophytis are efficient siderophore producers. The isolates of *Methylobacterium* spp. producte. hydroxamate-type, siderophores. The growth of plant was stimulated by the presence of siderophores of the of endophytic *Methylobacterium mesophilicum*.(Lacava *et al.*, 2014). *Azotobacter vinelandii*, *Bacillus megaterium*, *Bacillus subtilis*, *Pantoea allii* and *Rhizobium radiobacter* produced maximum siderophore in stationary phase, while *A. vinelandii* (at 72 h) showed maximum production during exponential phase of the growth. The presence of catechol-type siderophores for *B. subtilis* and *R. radiobacter* and hydroxamate-type siderophores for *B. megaterium* and *P. allii*. was determined in culture filtrates while *A. vinelandii*, contains both catechol and hydroxamates types siderophore. The highest iron-chelating capacity, was noticed by *B. megaterium* followed by *B. subtilis* and *A. vinelandii* at pH 9.0. (Ferreira *et al.*, 2019). The modified microplate method for siderophore production can be used for both qualitatively and quantitatively, making it less tedious, and cheaper method to screen PGP character of plant-associated bacteria. (Arora *et al.*, 2017).

Catalase an enzyme converts hydrogen peroxide to water and oxygen is important to protecting the cell from oxidative damage by reactive oxygen species (ROS). The endophytic bacterial isolates IPNR, IPNL, IPKR and IPKL showed catalase activity with maximum activity IPNR isolate. The presented amount of catalase in usually considered as stress tolerant characteristic.

The maximum phosphate solubilizing activity with

decrease in the pH of the medium on the 6th day of incubation (DAI) was detected in *Bacillus subtilis* (LP31 L03) in decreasing order TCP, FePO₄ and AlPO₄. The endophytic bacteria with phosphate solubilizing activity have potancial in biofertilizer technology. (Borah *et al.*, 2017). Phosphate-solubilizing bacteria (PSB) as inoculants have the ability to convert insoluble forms of iron and aluminum phosphates in acidic soils and calcium phosphates in alkaline soils. (Walia *et al.*, 2017). The isolates of *Aneurinibacillus* sp. and *Lysinibacillus* sp. solubilized phosphate in liquid medium supplemented with tricalcium phosphate. The isolates exhibiting variations in their capacity to solubilize P significantly reduced the pH of the liquid medium and exhibited acid phosphatase activity. (Matos *et al.*, 2017).

In this present study all the endophytic bacterial isolates showed an appreciable amount of potash and zinc solubilization activity. The maximum potash and zinc solubilization activity was in IPNR and IPKR.

The zinc solubilizing rhizobacteria isolated from sugarcane, screened for zinc solubilizing ability on five different insoluble zinc sources. *Pseudomonas fragi*, EPS 6 *Pantoea dispersa*, EPS 13 *Pantoea agglomerans*, PBS 2 *E. cloacae* and *Rhizobium* sp. were superior based on their zinc solubilizing potential (Kamran *et al.*, 2017). Out of the six promising Zn solubilizing bacteria *B. megaterium*, KY687496 was found to be the most potential strain owing to its enhanced Zn Solubilization with a marked decrease in pH due to enhanced gluconic acid production. (Dinesh *et al.*, 2018).

All the four endophytic bacterial isolates (IPKL, IPKR, IPNL and IPNR) were resistant to antibiotics Nalidixic acid and Ampicillin, isolates IPNR, IPKR and IPNL towards erythromycin and IPNR also showed resistance against Neomycin. The isolates were sensitive to Ciprofloxacin, Streptomycin, Kanamycin, Tetracycline and Rifampicin antibiotic.

Antibacterial activity test showed that *B. amyloliquefaciens* exhibited inhibition against MRSA and *K. pneumonia* bacteria, while *Bacillus* sp.1 and *Bacillus* sp.2 against *K. pneumoniae* bacteria. (Indrawati *et al.*, 2018, *Bacillus subtilis*, *Penicillium chrysogenum* and *Streptomyces* sp were checked for antibacterial activity against *Staphylococcus aureus* (ATCC 29213), *Pseudomonas aeruginosa* (ATCC 27853), *Escherichia coli* (ATCC 25922) and *Klebsiella pneumonia* (ATCC 4352). *Penicillium chrysogenum* metabolites showed maximum

antimicrobial activity against all the last strains. *Bacillus subtilis* metabolites showed activity against *Staphylococcus aureus* and *Pseudomonas aeruginosa* whereas *Streptomyces* sp. showed minimum activity against all the four tested organisms. (Sethi *et al.*, 2013). Antibiotic activity of the endophytic bacterial strains may help in the identification of strains.

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