

International Journal of Innovative Research in Science, Engineering and Technology

(An ISO 3297: 2007 Certified Organization)

Vol. 4, Issue 8, August 2015

A Study on Plant Growth Promoting Activity of the Endophytic Bacteria Isolated from the Root Nodules of *Mimosa Pudica* Plant

Nivya R. M.

PG Student, Department of Biotechnology and Biochemical Engineering, Sree Buddha College of Engineering,
Pattoor, Alappuzha, Kerala, India.

ABSTRACT: Plant growth promoting endophytic rhizobacteria are usually involved with host plants in mutual interaction. They promote plant growth directly or indirectly, via production of phytohormones, biocontrol of host plant diseases or improvement of plant nutritional status. Here through this study, a bacterial strain was isolated from surface-sterilized root nodules of *Mimosa pudica*; which was screened through phenotypic, biochemical and physiological characterisation, in which the isolate was found to be Gram-negative, motile, capsulated, non-endospore forming rod with catalase production, acid production and starch hydrolysing ability. The isolate was also found to utilize carbon sources like mannitol, sucrose, glucose and galactose. Further the isolate was studied for determining its plant growth promoting activity, by studying its biostimulating, biofertilising and biopesticidal activity. In each study the isolate showed a better result suggesting its significance in use as either a biostimulant, biofertiliser or a biopesticide as a sustainable agricultural approach.

KEYWORDS: Endophytic rhizobacteria, phytohormones, *Mimosa pudica*, biostimulant, biofertiliser, biopesticide, sustainable agricultural approach.

I. INTRODUCTION

The application of beneficial bacteria to the soil for the promotion of plant growth and the biological control of soil-borne pathogens is considered as a general technique since earlier days and nowadays biotechnology has opened up new possibilities concerning this aspect. Since the large scale release of genetically engineered bacteria to the environment faces a number of regulatory hurdles; the need to isolate and select superior, naturally occurring rhizosphere bacteria continues to be the subject of interest.

Plant-associated bacteria that live inside plant tissues without causing any harm to plants are defined as endophytic bacteria. Endophytic bacteria reside within plant tissues and have often been reported to promote plant growth. Plant growth promoting rhizobacteria (PGPR) are a group of bacteria that can be found in the rhizosphere, in association with roots which can enhance the growth of plant through their direct and indirect mechanisms as plant growth promoters and biological control agents. Direct mechanisms by PGPR, include the provision for bioavailability of phosphorus for plant uptake, nitrogen fixation for plant use, sequestration of iron for plant by siderophores, production of plant hormones and lowering plant ethylene levels using ACC deaminase that accumulate during biotic and abiotic stresses. Indirect mechanisms of PGPR include production of phytohormones, production of antibiotics, reduction of iron available to phytopathogens in the rhizosphere, induction of systemic resistance against various pathogens and pests in plants etc. These activities should be evaluated from their effects on plant performance and yield under agricultural settings for developing them as a proper plant growth promoter. PGPRs are involved with host plants in mutual interaction. Here in this study a bacterial strain was isolated from the root nodules of *Mimosa pudica*, a herb that shows sensation on touch commonly known as touch-me-not. And the isolate was found to have biostimulating, biopesticidal and biofertilising activity in test plants used.

International Journal of Innovative Research in Science, Engineering and Technology

(An ISO 3297: 2007 Certified Organization)

Vol. 4, Issue 8, August 2015

II. RELATED WORK

From the early practices onwards the microorganisms interacting with plants are known for their significant role in plant growth promotion especially those which reside inside the root nodules of plants. The first clear indication of improved plant growth and biological control of root pathogens due to seed bacterization with Rhizobacteria were reported in studies which showed the plant growth promoting effects of *Pseudomonas* strains which were antagonistic to a wide range of plant pathogens *in vitro* [3]. Five genera of rhizobia, namely *Rhizobium*, *Sinorhizobium*, *Azorhizobium*, *Bradyrhizobium* and *Mesorhizobium*, had been identified in *Bergey's Manual of Systematic Bacteriology* [7]. These bacteria have been studied as plant growth promoters for increasing agricultural production and as biocontrol agents against plant diseases. The term plant growth promoting rhizobacteria (PGPR) was then coined to include bacteria inhabiting the root and rhizosphere soil which have the ability to increase plant growth [6]. Later on different studies the biocontrol activity of certain PGPRs were reported. In a study, significant growth inhibition of *Sclerotium rolfisii* by *Pseudomonas* BRL-1 in its dual culture was reported [11]. The biocontrol potential of fluorescent *Pseudomonas* isolated from rhizosphere of pea and wheat *in vitro* and *in vivo* against maize sheath blight caused by *Rhizoctonia solani* were studied and found out that fluorescent *Pseudomonas* are diverse with respect to their biocontrol potential [12]. The mechanisms of growth promotion by these PGPR are complex and appear to comprise both changes in the microbial balance in the rhizosphere and alterations in host plant physiology [5]. It was reviewed that the PGPRs have the ability to produce IAA a phytohormone which is known to be involved in root initiation, cell division and cell enlargement hormone and implicated it in the growth promotion by PGPR. The study demonstrated that bacterial IAA from *P. putida* played a major role in the development of host plant root system [9]. These studies clearly indicate the PGPR have diverse mechanism for the growth promotion of plants and work efficiently in both greenhouse and field conditions.

A study on *M. pudica* plants showed that endophytic bacteria reside within these plant tissues and have been reported to promote plant growth [10]. Further study on these bacteria can be helpful in modifying these bacteria significantly and can be introduced with the planting material as a plant growth promoter.

III. MATERIALS AND METHODS

1. Isolation of bacteria

a. Sample collection: Plants of *M. pudica* were uprooted for their root nodules. Root was separated and washed several times. Nodules were collected from the root and were washed with distilled water.

b. Surface Sterilization: Nodules were collected and washed several times in sterile water. The nodules were then surface-sterilized by dipping them on 70% ethanol and 0.1% HgCl₂ for one minute and then they were repeatedly washed with sterile water.

c. Sample preparation: Sterile nodules were then crushed. The resulting suspension was serially diluted. Serial dilution was done up to 10⁻⁷ dilution. Resulting sample suspension of 10⁻¹, 10⁻⁶ and 10⁻⁷ dilution were streaked on yeast extract mannitol agar (YEMA) plates, and they were incubated at 28C for 48 hrs.

d. Bacteria isolation: Isolated colonies were picked after 48 hrs of incubation from the YEMA Plates. Culture was then maintained in YEMA slant.

2. Bacterial characterization

After 48 hrs of incubation single colonies were picked for further characterization. Bacteria were phenotypically, biochemically and physiologically characterized.

a. Phenotypic Characterization: Phenotypic characterization of the isolate is done. It was done for gram reaction (Hucker's method), mobility test, Capsule staining (Gin's Method) and endospore staining.

b. Biochemical Characterization: It is done for determining the biochemical characteristics of the isolate.

(i) **Catalase production test** done based on slide drop method using hydrogen peroxide.

(ii) **Acid production test** was done to determine the ability of the isolate to produce by observing the change in pH before and inoculation of the isolate. The indicator used here is Bromothymol Blue 5.

(iii) **Starch Hydrolysis Test** In this test; starch agar is inoculated with the isolate. After incubation at an appropriate temperature, iodine was added to the surface of the agar. Iodine turns blue-black in the presence of starch. Bacteria

International Journal of Innovative Research in Science, Engineering and Technology

(An ISO 3297: 2007 Certified Organization)

Vol. 4, Issue 8, August 2015

which show a clear zone around the growth produce the exoenzyme amylase which cleaves the starch into di- and monosaccharide. So, this test is to identify whether the isolated bacteria hydrolysis starch or not.

c. Physiological Characterization: Different carbon source utilization and growth cycle is determined.

(i) **Determination of Utilization of Different Carbon Source:**-The ability of the isolate to utilise different carbon sources was determined. Determination was done using the indicator Bromothymol blue which shows acid or alkali production indicating carbon source utilization. Basal liquid medium (BLM) is used for determining carbon source utilization by the isolate. Different carbon sources used were Mannitol, Sucrose, Glucose, Galactose, and Maltose.

BLM with different C sources were inoculated with isolate. It was then kept for incubation @ 28 C for 4 days. After 4 days of incubation the flasks were observed for any color change.

(ii) **Growth Curve Determination:** - Isolate has been inoculated to YEM broth and was kept on shaking incubator at 150 r.p.m and absorbance was taken for equal time intervals from zeroth time onwards at 610 nm where uninoculated broth was kept as blank.

3. Study on Plant Growth promoting activity of the Isolate

Now the isolated bacteria from the root nodules *M. pudica* was taken for studying its plant growth promoting activity. Plant growth promoting activity is studied for its determining its; **Biostimulant Activity, Biofertilization Activity, and Biocontrol Activity**

a. Study on Biostimulant activity: In this the ability production of phytohormones indole acetic acid (IAA) by the isolate was studied.

i) Detection of IAA production: It was done in 50 ml of Nutrient broth (NB) containing 0.1% D- tryptophan inoculated with 500 µl of 24 h old bacterial cultures and incubated in refrigerated shaking incubator at 30±0.1 °C and 180 rpm for 48 h in dark. The bacterial cultures were centrifuged at 10,000 rpm for 10 min at 4 °C after incubation and supernatant was taken and mixed with Salkowski's reagent in ratio 1:4 and absorbance of the resultant pink color was read after 30 min at 535 nm in UV/Visible Spectrophotometer.

ii) Extraction of IAA: Bacteria were cultured in NB amended with different concentration (1mg/ml, 5mg/ml) of tryptophan for 1 week. After the growth period culture was centrifuged and supernatant was taken. 50ml of supernatant was acidified to pH 2.5. Extracted twice with ethyl acetate @ double volume of supernatant and was evaporated to dryness. Extract was dissolved in methanol at different dilutions.

iii) Thin Layer Chromatography of IAA: TLC of IAA was performed in which TLC plates were prepared with silica gel G and were spotted with the IAA extract. Solvent used was ethyl acetate: chloroform: acetic acid (55:35:10). Whole setup was left for capillary run and plates were allowed to dry. Salkowski's reagent was sprayed to get pinkish band.

b. Study on Biofertilization activity: Ability of isolate to fix the atmospheric nitrogen is determined.

i) Nitrogen Fixation ability (Production of ammonia): Bacterial cultures were tested for the production of ammonia in peptone water. Freshly grown cultures were inoculated in 10 ml peptone water and incubated for 48–72 h at 30°C. After incubation Nessler's reagent (0.5 ml) was added in each tube.

c. Study on Biofertilization activity under Green house: Seedlings were surface sterilized. They were then subjected to six washings with sterile distilled water. Thoroughly washed seedlings were grown on pots under green house. 2 ml of bacterial suspension were applied on seedlings present in each pot with the sterilized pipette after wounding the roots using sand paper. The control group seedlings were grown without adding the bacterial suspension. The plants were kept for growth for 3-4 weeks; the seedlings were then examined for shoot, root growth (length, fresh weight). And the results were comparing with control for analysis. Here seedlings of two plants are used: tomato seedlings and mustard seedling.

d. Study on Biocontrol activity: Ability of isolate to produce HCN is determined which can act against plant pathogens.

i) Hydrogen cyanide production assay: Screening of bacterial isolates for hydrogen cyanide (HCN) production was done as per methodology described by Castric. HCN production potential of the bacteria was assessed as per the following scoring.

No color change: No HCN production, Brownish coloration: Weak HCN production, Brownish to orange: Moderate HCN production, Orange to reddish brown: Strong HCN production

e. Study on Biocontrol activity on Pot experiments: Sterilized seedlings were sown on pots in green house after inoculating with pathogen. Pathogen used is *Fusarium culmorum*. Test plants were applied with isolate culture solution along with pathogen and were allowed to grown under optimum conditions for 3-4 weeks. After that the plant were detected for the presence of discoloration, galls, curls etc. Then plants were harvested and plant extracts were taken

International Journal of Innovative Research in Science, Engineering and Technology

(An ISO 3297: 2007 Certified Organization)

Vol. 4, Issue 8, August 2015

after proper sterilization for the analysis of extract for the presence of different enzymes and phenol content determination which shows the extend of pathogen attack.

(i) **Analysis of plant extract:** Extract was weighed and taken for homogenization with Phosphate buffer pH 7.0 and centrifuged @ 10000 r.p.m for 20 mins. Afterwards supernatant was collected and it was taken for the analysis. Here activity of peroxidase and estimation of total phenolic content in the plant extract was determined.

(ii) **Assay of peroxidise:** The reaction mixture consisted of 2.5 ml of a mixture containing 0.25 per cent (v/v) guaiacol in 0.01M. Phosphate buffer, pH 7.0 and 0.1 M hydrogen peroxide. Enzyme extract (0.1 ml) was added to initiate the reaction which was followed calorimetrically at 470 nm. Crude enzyme preparations were diluted to give changes in absorbance at 470 nm of 0.1 to 0.2 absorbance units/min. Boiled enzyme was used as blank. Activity was expressed as the increase in absorbance at 470 nm $\text{min}^{-1} \text{mg}^{-1}$ of protein.

$$\text{Enzyme activity in units /Litre} = (3.18 \times 0.1 \times 1000) / (6.39 \times 1 \times \Delta t \times 0.1)$$

(iii) **Estimation of total phenol content:** One ml of the plant extract was added to 5 ml of distilled water and 250 μl of Folin-Ciocalteu reagent (1N) and the solution was kept at 25°C. After 3 min one ml of saturated solution of sodium carbonate and one ml of distilled water was added and the reaction mixture was incubated for 1 hr at 25°C. The absorption of the developed blue colour was measured using UV-visible spectrophotometer at 725 nm. The content of the total soluble phenols was calculated according to a standard curve obtained from a Folin-Ciocalteu reagent with a phenol solution and expressed as catechol- equivalents /mg tissue weight.

IV. RESULTS AND DISCUSSIONS

The nodules were crushed serially diluted and streaked on to YEMA plates. After incubation of 48 hrs isolated colonies were seen on YEMA plates as shown in the figure.

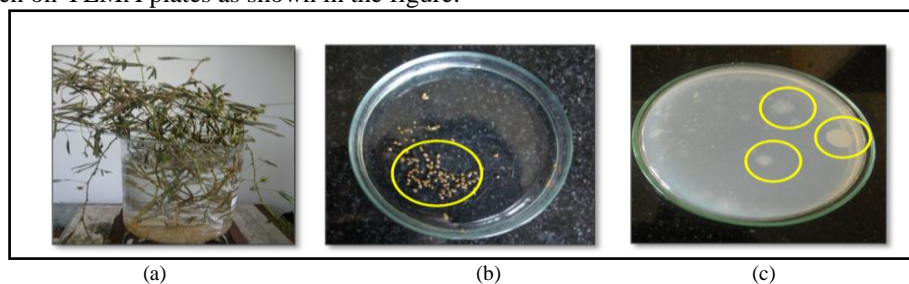


Fig 1: The figure shows (a) The *M.pudica* plant, (b) nodules that were collected from its roots and (c) the isolated colonies on YEMA plates. The isolated bacteria were then subjected for characterization. The bacteria were gram negative, capsulating, motile rod having ability of Catalase and acid production. It could hydrolyze starch and also found to utilize various carbon sources. The observations of phenotypical, biochemical and physiological characterization can be summarized as:

Characterization	Observation and result
1. Phenotypic	
(i) Shape	Rod shaped
(ii) Grams reaction	Gram negative
(iii) Motility test	Motile bacteria
(iv) Capsule staining	Capsulating
(v) Endospore staining	non endospore forming
2. Biochemical	
(i) Catalase test	Catalase producing(rapid formation of gas bubbles were observed)

International Journal of Innovative Research in Science, Engineering and Technology

(An ISO 3297: 2007 Certified Organization)

Vol. 4, Issue 8, August 2015

(ii) Acid production test	Acid producing (Yellow colored zone was seen on plates)
(iii) Starch hydrolysis test	Starch hydrolysing(clear zones were seen on plates)
3. Physiological	
(i) Determination of C source Utilisation	
a) Mannitol	Mannitol utilising (color change is observed from Green to Yellow)
b) Sucrose	Sucrose utilising(color change is observed from Green to Yellow)
c) Galactose	Galactose utilising(slight color change is observed from Green to Yellow)
d) Glucose	Glucose utilising(slight color change is observed from Green to Yellow)
e) maltose	Not utilising(color change is not observed from Green to Yellow)

Table 1: Phenotypic, Biochemical and physiological characterization of Isolate

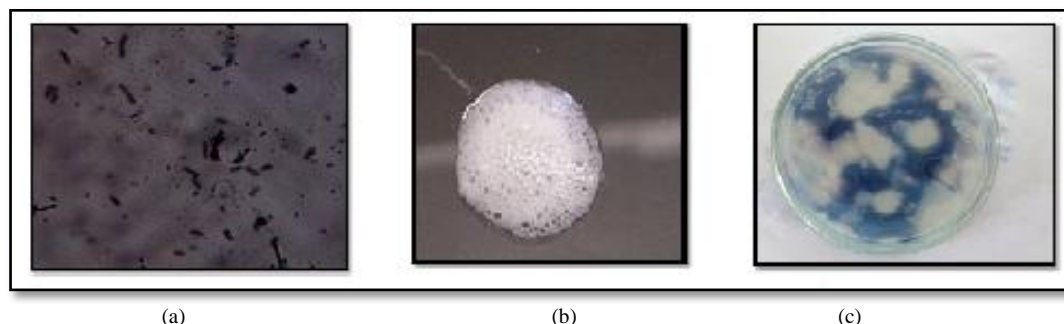


Fig 2: The figure shows observation of isolate as (a) gram negative, (b) Catalase positive and (c) Starch hydrolyzing

The isolate was checked for its carbon source utilization test for mannitol, sucrose, glucose, Galactose and maltose in which isolate was found to utilize all carbon source except maltose.

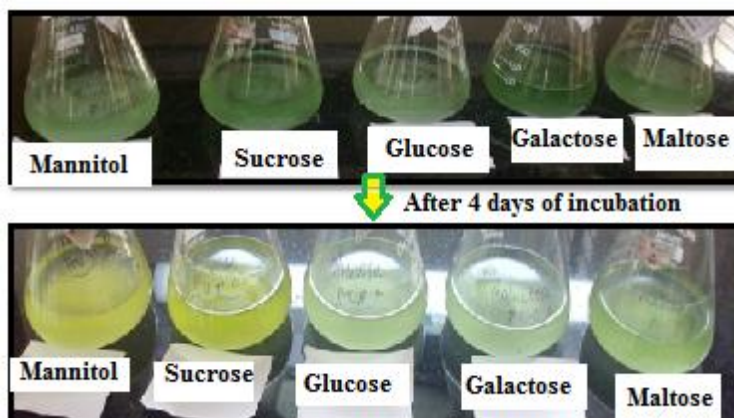


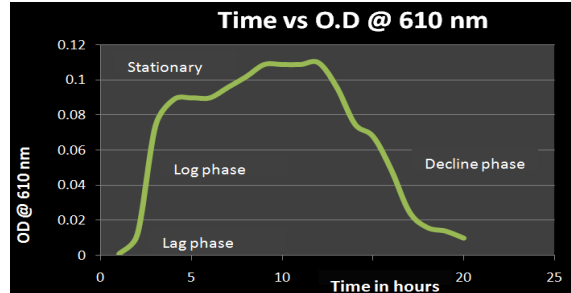
Fig 3: Observation on C-source utilization test. Change in color from green to yellow of medium shows that the isolate has utilized the carbon source and converted it to acids.

For growth curve determination absorbance value for equal time intervals were determined and the growth curve was plotted as:

International Journal of Innovative Research in Science, Engineering and Technology

(An ISO 3297: 2007 Certified Organization)

Vol. 4, Issue 8, August 2015



Graph 1: Growth curve of the Isolate.

The isolate was then evaluated its plant growth promoting activity. At first its biostimulant activity was checked. The ability of the isolate to produce Indole acetic acid was determined and was observed that the isolate showed the ability to produce Indole acetic acid on Nutrient broth (NB) containing 0.1% DL- tryptophan. After incubation of 48 hours in dark the 1 ml of supernatant broth added with 4 ml of salkowski’s reagent showed a change in color from yellow to light red.

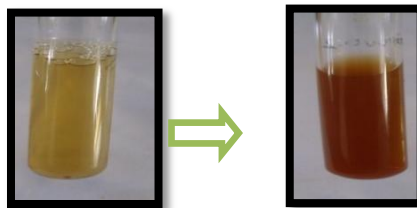
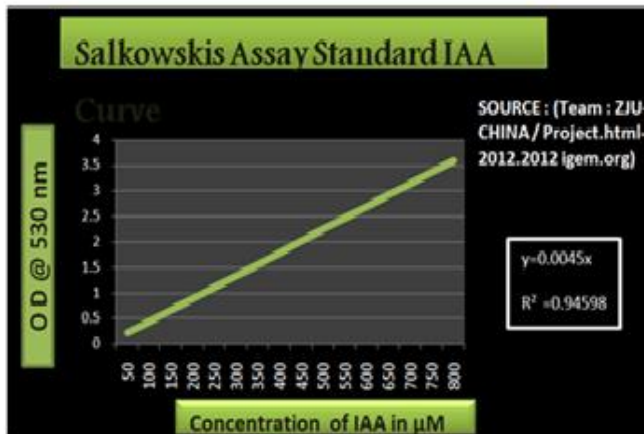


Fig 4: Change in color of medium after incubation with isolate from yellow to red after the addition of Salkowskis reagent indicates the production of IAA.

For IAA detection isolate was grown in NB with different concentration of Tryptophan (1 mg/ml, 2mg/ml, 5mg/ml).After which it was centrifuged and OD @ 530 nm was read for supernatant IAA concentration was estimated from standard graph for corresponding OD as given in table.



Graph 2: Standard IAA concentration vs. OD@530nm curve

Sl no:	Concentration of tryptophan in medium	OD @ 530nm	Concentration of IAA from Std graph (μM)
1	1 mg/ml	1.51	335.55
2	2 mg/ml	2.407	534.88
3	5 mg/ml	3.362	747.11

Table 2: Concentration of IAA corresponding to A₅₃₀ of samples

The IAA concentration in the sample can be determined by extrapolating the OD value of sample in the standard curve to x-axis.

The IAA which was extracted from the isolate was checked in TLC in which the pink bands after spraying with the Salkowski’s reagent showed its presence.

International Journal of Innovative Research in Science, Engineering and Technology

(An ISO 3297: 2007 Certified Organization)

Vol. 4, Issue 8, August 2015



Fig 5: Pink bands in the TLC plate shows the presence of IAA

Next the isolate was checked for its biofertilizing activity. The ability of isolate to fix atmospheric nitrogen was checked by peptone water assay and peptone water inoculated with isolate after incubation of 72 hours when added with Nessler’s reagent showed a color change to brownish orange indicating the presence of ammonia.

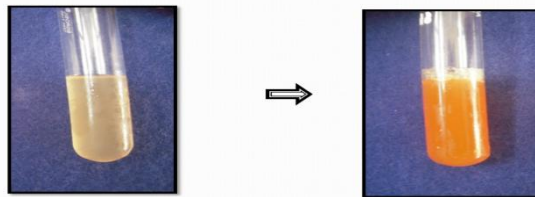


Fig 6: Color change of peptone water to orange shows the presence of ammonia indicating ability of isolate to fix atmospheric nitrogen.

The biofertilizing activity of the isolate was then determined under green house. Both tomato and mustard plant which were used as test plants showed increased growth properties. All parameters like shoot length, root length, fresh weight, area of leaves showed significant increase in growth when compared to control plants as given in table 3 and 4.

Sample (Tomato)	Shoot length (SL)	Root length (RL)	Fresh Weight (FW)	Dry Weight (DW)	No:of Leaves (NL)	Area of Leaf (AL)
Control	12.5 cm	4.2 cm	1.556 g	0.7 g	27	4.35 cm ²
Test	43.7 cm	7.7 cm	6.69 g	0.772 g	68	5 cm ²

Table 3: Table showing the Biofertilization activity of isolate on tomato plant by comparing various parameters of control and test plants

Sample (Mustard)	Root length	Shoot length	Fresh wt	Dry wt	No: of Leaves
Control	4.2cm	6.2 cm	0.116 g	0.0094 g	4
Test	5.1 cm	8.5 cm	0.445 g	0.023 g	8

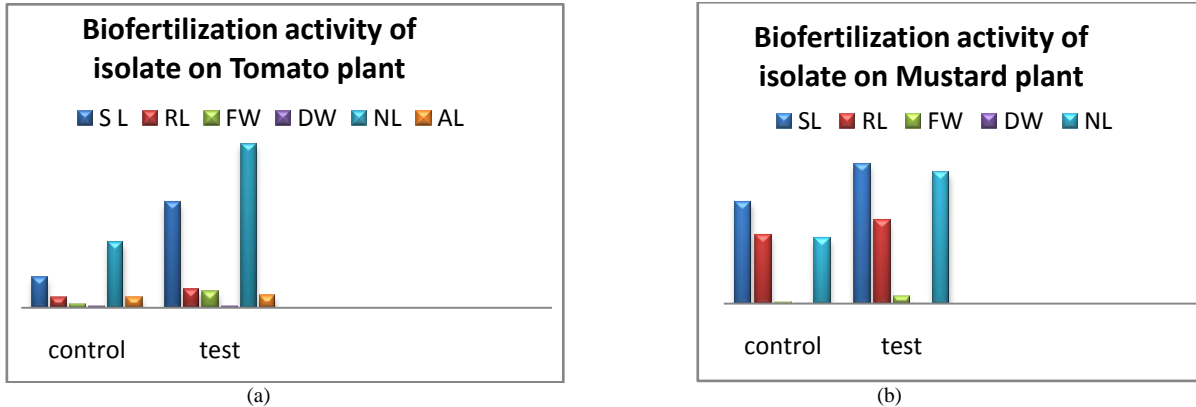
Table 4: Table showing the Biofertilization activity of isolate on mustard plant by comparing various parameters of control and test plants

The biofertilization activity shown by both plants as given in table can be graphically represented as given below:

International Journal of Innovative Research in Science, Engineering and Technology

(An ISO 3297: 2007 Certified Organization)

Vol. 4, Issue 8, August 2015



Graph 3: Graphical representation of Biofertilization activity on (a) Tomato plant, and (b) Mustard plant

The isolate was then checked for its biocontrol activity and showed a good biocontrol activity against the test pathogen *Fusarium culmorum*. It showed change in color from yellow to orangish brown in HCN production test plates indicating it to be a moderate HCN producer.

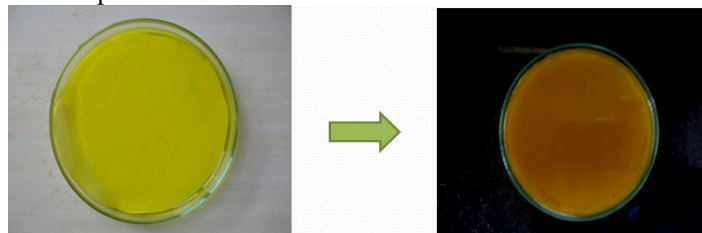


Fig 7: Color change to orangish brown shows the isolate is a moderate producer of HCN.

At last the biocontrol activity of isolate was checked under green house. The plants co-inoculated with the isolate along with the pathogen showed lesser pathological attack like yellowing, curling of leaves and rotting of roots etc., when compared to those inoculated with pathogen alone.

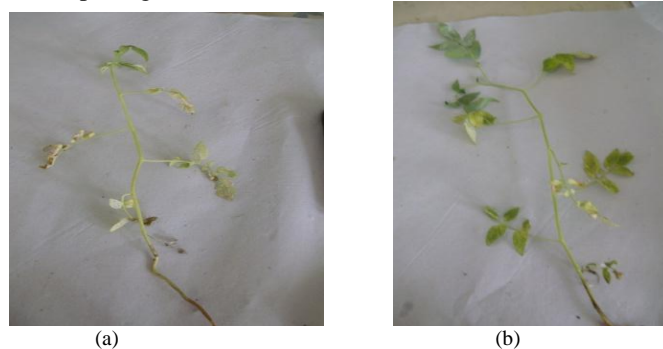


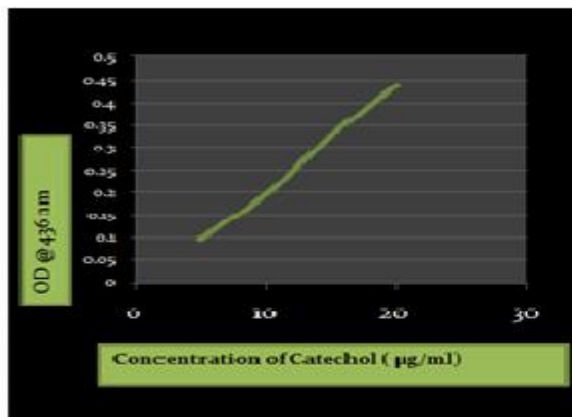
Fig 8: Observation of plant extract for determining biocontrol activity of isolate by comparing (a) Tomato plant inoculated with pathogen alone and (b) Tomato plant co-inoculated with the isolate and pathogen

Phenol content assay on plant samples showed that in both mustard and tomato; the plants treated with isolate showed an increased amount of phenolic content. The phenolic concentration has been estimated from standard graph and expressed as catechol- equivalents mg^{-1} tissue weight.

International Journal of Innovative Research in Science, Engineering and Technology

(An ISO 3297: 2007 Certified Organization)

Vol. 4, Issue 8, August 2015



Graph 3: Standard Catechol concentration vs OD@436nm curve

Sl no:	OD @ 436 nm	Concentration from std graph catechol equivalence / 0.75 mg of plant extract.
Mustard control	0.635	28.5 µg/ml
Mustard test	1.023	46 µg/ml
Tomato control	0.49	22.5 µg/ml
Tomato test	0.611	27.5 µg/ml

Table 5: Concentration of Catechol corresponding to A₄₃₆ of samples

The catechol concentration in the test sample can be determined by extrapolating the OD value of test sample in the standard curve to x-axis.

While checking the activity of enzymes peroxidase in the test plant samples, increased activity of peroxidase was seen in tomato plant treated with isolate when compared to control; while in mustard plant the activity observed was comparatively less.

Sl no:	Δt (sec)	Enzyme activity in units/ L
Mustard control	90	5.52
Mustard test	60	8.492
Tomato control	45	11.05
Tomato test	15	33.17

Table 6: Table showing the peroxidase enzyme activity in both test plants when compared to their respective controls.

The test plants, tomato plants and mustard plants along with control were grown under optimum condition of temperature and sunlight in green house for a period of 3-4 weeks and observed the above results.



(a) (b)
Fig 9: Test plants, (a) Mustard plants and (b) Tomato plants, under green house.

International Journal of Innovative Research in Science, Engineering and Technology

(An ISO 3297: 2007 Certified Organization)

Vol. 4, Issue 8, August 2015

V. CONCLUSIONS

New developments occurring in the field of biotechnology and agricultural microbiology with a view for sustainable agriculture is getting more attraction these days. With the problems related to release of genetically modified microorganisms had made the necessity of developing native microorganism as a better option. In this study such a bacterium has been isolated from the root nodules of *Mimosa pudica* plant. The isolated bacterium was an endophytic, gram negative, bacillus bacteria and it was found to have a lot of plant growth promoting activity. It showed positive IAA, HCN production. It also showed inhibiting activity towards the fungus *Fusarium culmorum* (test pathogen used). Experiments on plant for its Biocontrol and Biofertilisation activity had comparatively (with control) positive results on both Mustard and Tomato plants .In which tomato plants showed better results indicating the effect of the isolate on the plant.

REFERENCES

- [1]. Anderson, G.K. Beveridge, T. J., and H. C. Clark., "Chemical Mechanism of the gram stain and synthesis of a new electron-opaque marker for electron microscopy which replaces the iodine mordant of the stain." *Journal of Bacteriology*. 156 (2):837-845, 1983.
- [2]. Atlas, R. M. (ed.), "Handbook of microbiological media for the examination of food." 2nd ed. CRC Press/Taylor & Francis, Boca Raton, FL. 2006
- [3]. Burr, T.J., Schroth, M.N. and Suslow, T.V., "Increased potato yields by treatment of seed pieces with specific strains of *Pseudomonas fluorescens* and *P. putida*". *Phytopathology*, 68: 1377-1383, 1978.
- [4]. Gerhardt, P., R. G. E. Murray, R. N. Costilow, E. W. Nester, W. A. Wood, N. R. Krieg, and G. B. Phillips. "Manual of methods for general microbiology." *ASM Press*, Washington DC, 1981.
- [5]. Glick, B.R., Patten, C.L., Holguin, G. and Penrose, D.M., "Biochemical and Genetic Mechanisms used by Plant Growth Promoting Bacteria". *Imperial College Press*, London, Frankenberger WT, pp.125-140, 1999.
- [6]. Kloepper, J.W. and Beauchamp, C.J., "A review of issues related to measuring colonization of plant roots by bacteria." *Canadian Journal of Microbiology*, 38: 1219-1232, 1992.
- [7]. Krieg, N.R. and Holt, J.G., "*Bergey's Manual of Systematic Bacteriology*." Williams and Wilkins, Baltimore and London, 1984.
- [8]. McLeod, J. W., and J. Gordon.. "Catalase production and sensitiveness to hydrogen peroxide amongst bacteria: with a scheme for classification based on these properties." *Journal of Pathology and Bacteriology*. 26:326-33, 1923
- [9]. Peck, S.C. and Kende, H., "Sequential induction of the ethylene biosynthetic enzymes by indole-3-acetic acid in etiolated peas". *Plant Mol. Biol.*, 28: 293-301, 1995.
- [10]. Piyush Pandey, S. C. Kangand D. K. Maheshwari, "Isolation of endophytic plant growth promoting *Burkholderia* sp. MSSP from root nodules of *Mimosa pudica*." revised accepted 11 March 2005
- [11]. Surjit Sen, Tanaya Bose, Kinnari Basu, Rupa Acharya, Nirmalendu Samjapati and Krishnendu Acharya, "*In vitro* antagonistic effect of fluorescent pseomonas BRL-1 against *Sclerotium rolfsii*". *Indian journal of Phytopathology*, 59: 227-230, 2006.
- [12]. Tripathi, M. and Johri, B.N., "In vitro antagonistic potential of fluorescent pseudomonads and control of sheath blight of maize caused by *Rhizoctonia solani*." *Indian Journal of Microbiology*, 42: 207-214, 2002.