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PLANT GROWTH PROMOTING AND PHYTOSTIMULATORY POTENTIAL OF *Bacillus Subtilis* AND *Bacillus Amyloliquefaciens*

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ABSTRACT

Root colonizing bacteria that exert beneficial effects on plant growth have been defined as plant growth promoting rhizobacteria (PGPR). The present study aimed to the production of indole-3-acetic acid (IAA) and siderophore in WR-W2 and MR-AI strains under *in vitro* condition. Synthesis of IAA and siderophore was checked in presence of different carbon sources in $JNFb^-$ medium amended with L-tryptophan (100 µg/ml) and iron limited M9 medium, respectively. Time course study and simultaneous production of IAA and siderophore was performed in the strain MR-AI and WR-W2. Malate followed by acetate was found as the most suitable sole carbon source for both the IAA and siderophore production by the strain. Siderophore production was inhibited above 20µM concentration of Fe (III). Catecholate type of siderophore production was identified in the culture supernatant of both the strain. Furthermore, significant increase in rice plant (*Pusa sugandha III*) growth response was observed, when the strains were used as bioinoculum under gnotobiotic condition, in presence of L-tryptophan (100µg/ml). 16S rRNA gene sequence reveals strains MR-AI as *Bacillus amyloliquefaciens* and WR-W2 as *Bacillus subtilis*. Strain WR-W2 was found to be more efficient as compared to MR-AI in terms of its capability to produce IAA and siderophore. Therefore, strain MR-AI and WR-W2 could be further applied as bioinnoculum in different formulation in seeds or crop fields for sustainable agricultural systems.

Keywords: indole-3-acetic acid, plant growth promoting rhizobacteria, *Bacillus subtilis*, *Bacillus amyloliquefaciens*, siderophore, tryptophan.

INTRODUCTION

Over the last decade, a significant research interest has been generated around PGPR, because of their capacity to increase plant growth, through various direct/indirect mechanisms [1]. Plant growth-promoting rhizobacteria (PGPR) are free-living, root colonizing, soil-borne bacteria exert phyto-stimulatory actions, when applied to seeds or crops by a combination of physiological attributes [2]. Plant beneficial activity involves N_2 fixation, [3] production of IAA and siderophore [4, 5]. Enhancement of plant growth by root-colonizing *Bacillus* sp. is well documented [6, 7, 8]. Studies reveal that *Bacillus* species are among the most prominent bacteria found to colonize plants root and soil populations [9]. The genus *Bacillus* is characterized by Gram positive, aerobic or facultative anaerobic, rod shaped bacteria that form endopores [10]. Moreover, common physiological traits important to their survival include production of a multilayered cell wall structure, formation of stress resistant endospore and secretion of peptide antibiotics, peptide, signal molecules and extracellular enzymes [11]. Quantitative and qualitative relation in these traits allow for these bacteria to inhabit diverse niches in agro ecosystems. Their microscopic size and omnipresence in soil facilitate their colonization of plants. The plant root colonizing *B. amyloliquefaciens* strain is a naturally occurring isolates, distinguished from the model organism *B. subtilis* by its abilities to stimulate plant growth [12]. The ability of *B. amyloliquefaciens* to efficiently colonize surfaces of plant root is a prerequisite of phytostimulation and their capability to form sessile, multicellular communities (biofilm).

In liquid culture without shaking *B. amyloliquefaciens* forms robust pellicle at the liquid air interface, whereas domesticated *B. subtilis* strains usually form thin fragile pellicles [13]. Furthermore, Plant growth promoting rhizobacteria, most of which are *Pseudomonas* and *Bacillus* sp. are applied to a wide range of agricultural species to enhance growth [14]. However, biological preparations from *Bacillus* sp. offer a biological solution to the formulation problem due to their ability to form heat and desiccation resistant spores [15]. Among Gram positive PGPR such as *Paenibacillus* relatively little is known about the beneficial traits *Bacillus* sp. which were originally considered as typical soil bacteria [8]. It has been estimated that more than 100 million tones of nitrogen, potash and phosphate-chemical fertilizers have been used annually in order to increase plant yield. The potential negative effect of chemical fertilizers on the global environment and the cost associated with production has led to research with the objective of replacing chemical fertilizers with bacterial inoculants. Moreover, bioproduction of IAA and siderophore in Gram-negative bacteria has been well documented [16]. In contrast to Gram-negative PGPR, little is known about the mechanism(s) of IAA production and iron acquisition in Gram-positive bacteria, especially *Bacillus* sp. [17]. Synthesis of IAA by the Gram positive, phytopathogen *Rhodococcus fascians*, recently was reported [18]. The present study aimed to investigate IAA and siderophore production by *B. subtilis* and *B. amyloliquefaciens* under *in vitro* conditions as well as to study their effect on rice plant growth responses.

MATERIALS AND METHODS

Estimation of IAA and siderophore production

Rhizospheric soil samples were collected from maize and wheat crop plant field at Agricultural farm of Banaras Hindu University, Varanasi, India. To isolate rhizospheric bacteria, the adhering soil on the root was gently shaken in sterile Milli Q water (MQW) and followed standard method of Baarraquio *et al*. (2000) [19]. Bacteria representative of the predominant morphological types present on the plates were selected at random and purified on JNFb$^-$ minimal medium [3]. Which contained the following ingredients per liter: malate 5.0g, K_2HPO_4 0.6g, KH_2PO_4 1.8g, $MgSO_47H_2O$ 0.2g, NaCl 0.1g, $CaCl_2.2H_2O$ 0.2g, $Na_2MoO_4.2H_2O$ 0.002g pH 5.8. JNFb$^-$ medium exogenously supplemented with 100µg/ml of L-tryptophan. To check siderophore level isolates were cultured on iron free M9 medium [20]. Which constitutes per liter: glucose 2.0 g, Na_2HPO_4 6.0g, KH_2PO_4 3.0g, NaCl 0.50g, NH_4Cl 1.0g, $MgSO_4$ 2.0g, $CaCl_2$ 0.015g, pH 7.0. Quantitative estimation of IAA and siderophore in broth was carried out using Erleameyer flasks (50 ml) containing 10 ml of JNFb$^-$ and iron free M9 medium, inoculated in triplicate with the bacterial strain (100µl) innoculum with approximately upto 2 x 10^8 cfu ml^{-1}). 0.2 % of Iron chelating agent ethylene diamine dihydroxy-phenylacetic acid (EDDHA) was supplemented in the medium.

Production of IAA and siderophore in presence of carbon sources

The effect of carbon on IAA and siderophore production was tested on JNFb$^-$ and M9 medium respectively. In order to check the effect of carbon source on IAA and siderophore production, malate from the JNFb$^-$ and glucose from the M9 medium was replaced by eight desired carbon sources as shown. Production of IAA and siderophore was checked according to the colorimetric assay of Gordon and Weber (1951) [21] and Schwyn and Neiland (1987) [22], respectively. Culture was incubated in a temperature controlled shaker (Orbitek L T, Scigenics Biotech Pvt Ltd, Chennai, India) at 120rev/min at 30±2^0 C For IAA test, 1.0 ml culture supernatant was added with 2 ml of salkowaski reagent [23] constitutes 1.0 ml of 0.5 M $FeCl_3$ and 50 ml of 35% $HClO_4$. For siderophore test, 0.5 ml of culture supernatant was added with equal volume of CAS indicator reagent, contains: 6 ml of 10mM of HDTMA, 1.5 ml of 1mM $FeCl_36H_2O$ in 10mM HCl, 7.5ml of 2 mM chrome azurol-S, 10 ml of 4. 307g piperazene in 12 M HCl, pH was adjusted to 5.6 by conc. HCl in 100 ml MQW. Development of pink color indicates release of IAA and siderophore in the culture medium. Absorbance was recorded at 530nm for IAA and 630nm for siderophore using Milton Roy 1201 Spectronic UV-VIS spectrophotometer (Rochester, NY, USA). The concentration of IAA was estimated using a standard prepared with pure IAA (Sigma Aldrich, MO, USA). The level of siderophore was estimated using a standard prepared with 2, 3-dihydroxybenzoic acids (2-3 DHBA) (Sigma, USA).

Thin layer Chromatography of IAA and siderophore

To further check IAA and siderophore production strains were cultured in JNFb$^-$ and M9 medium respectively. The supernatant of the culture fluid was obtained by centrifuging the stationary phase cultures at 10,000g for 15 min and the pH was adjusted to 2.8 with 1 N HCl. The auxins from the acidified culture extracted with equal volumes of ethyl acetate [24] was evaporated to dryness and resuspended in 1/4th volume of the supernatant in ethanol. To detect IAA production TLC was performed in N-Butanol: Glacial acetic acid: DDW in ratio 12:3:5 and developed in salkowski reagent [23]. The solvent used for detection of siderophore was: N-Butanol: Glacial acetic acid: DDW in ratio of 12:3:5 [25]. After completion of running the plate was sprayed with the CAS solution and development of the pink spots was monitored.

Effect of Fe-EDTA and $FeCl_3.6H_2O$ on siderophore production

Qualitative detection of siderophore production was carried out as described by highly sensitive chrome azurol-S (CAS) assay of Schwyn and Neilands (1987) [22]. To detect siderophore production activity, strain MR-AI and WR-W2 was grown on CAS (Hi Media, India) added iron free M9 Agar-Agar medium. The culture medium was supplemented with Fe-EDTA (0, 10 and 20 µg/ml). 10 µl of 10^6cfu/ml was used for inoculation on the agar-agar plate. To further check effect of $FeCl_3.6H_2O$ on siderophore production, both the strain were grown in iron free M9 broth medium, containing $FeCl_3.6H_2O$ (0, 5, 10, 15 and 20 µM).

Identification of the type of siderophore production

To identify the type of siderophore production by the strain MR-AI and WR-W2, we followed the standard method of Arnow (1937) [26]. Both the strains were cultured in iron free M9 medium for 24h at 30±2^{0}C. 1.0ml of culture supernatant was added with 1.0ml of 0.5N HCl followed by addition of 1.0 ml nitrite-molybdate reagent. Thereafter, 1.0ml of 1N NaOH was added and volume was maintained to 5 ml with MQW. Appearance of bright red to pink color was considered positive for the presence of catecholate type of siderophore and absorbance at λ 500 nm was recorded. Control tube containing 1 µM $FeSO_4.7H_2O$.

Simultaneous production of siderophore and IAA under in vitro conditions

To further investigate simultaneous production of IAA and siderophore, strains were grown in iron free M9 medium, added with L-tryptophan 100µg/ml. Strain MR-AI and WR-W2 was inoculated (2 x 10^8 cfu ml-^{1}) in separate flasks in triplicate set. Estimation of both IAA and siderophore was performed from each of the culture flask. One set of control omitted with L-tryptophan was also prepared.

Inoculation of rice plant with MR-AI and WR-W2

Effect of MR-AI and WR-W2 on plant growth promotion was carried out in Fahreus carbon and nitrogen source free (FC^-N^-) [27] semisolid medium. Which contained the following ingredient per liter: $CaCl_2$ 0.10, $MgSO_4.2H_2O$ 0.12, KH_2PO_4 0.15, $Na_2HPO_4.2H_2O$ 0.15, Ferric citrate 0.005, Trace elements 1ml, ($Na_2MoO_4.2H_2O$ 0.05, $MnSO_4.H_2O$ 0.25, $ZnSO_4.7H_2O$ 0.07, $CuSO_4.5H_2O$ 0.0125, $CoCl_2.6H_2O$ 0.0119, H_3BO_4 0.003, DDW 100 ml) Agar 0.15% pH 7.0. Plant growth response was performed by the rhizobacterial strain, when used as bioinoculum both in the presence and absence of L-tryptophan (100µg/ml). Seeds of rice variety *Pusa sugandha-III* were dehulled and surface-sterilized with 70% ethanol for 2 min followed by treatment with 1% chloramine-T for 15 min. After thorough washing with sterilized double-distilled water (DDW), they were placed on Luria-Bertani (LB) agar plate and incubated at 30 ± 2^0C, until germination occurred. Seedlings free of any bacterial and fungal contamination were used for inoculation with MR-AI and WR-W2. Seedlings (1-2 cm long) were transferred in carbon and nitrogen free semisolid FC^-N^- medium in a culture tube (20 x 3.8 cm). Bacterial strain were grown in LB agar medium overnight and harvested by centrifugation. The pellets were washed twice with normal saline and resuspended in phosphate buffered saline (PBS). The seedlings grown in FC^-N^- medium were inoculated with bacterial suspension containing approximately 10^8 cells ml^{-1} and grown in a growth chamber [14h- light/10h-dark cycle at 27^0C and 25^0C (day-night, respectively)]. Plant growth parameter such as fresh root / shoot wt, root / shoot length as well as chlorophyll a) was recorded after 7 day of inoculation. Chlorophyll a content of one week grown rice plant was extracted in 80% (v/v) ethanol and absorbance was recorded at 663 and 645 nm.

PCR amplification

Whole cell genomic DNA was extracted following the standard protocol of [20] (Sambrook et al., 1989). The amplification of 16S rDNA was performed by the method of Eckert *et al*. (2001) [28]. Genomic DNA was subjected to 16S rDNA amplification using universal primer. Amplification of rDNA from pure cultures was performed in a final volume of 50 µl. The PCR reaction mix included; 1.5 U of Taq DNA polymerase (Banglore Genei, India), 1X PCR assay buffer with 25 mM $MgCl_2$, 20 pmol each forward and reverse primers ((Integrated DNA Technologies, Inc, CA, USA), each dNTPs was 200 µM (Banglore Genei, India) and 50 ng template DNA. Pair of primers used for amplification of 16S rDNA was, forward 5'-AGA GTT TGA TYM TGG CTC AG-3' and reverse 5`-CTA CGG CTA CCT TGT TAC GA-3`). Amplification was performed in PTC-100 Thermal Cycler (MJ Research, Inc, Walthon, MA, USA), using initial denaturation at 94^0C for 30 sec, annealing at 57^0C for 1 min, elongation at 72^0C and final extension at 72^0C for 5 min. and finally storage at 4^oC. 5 µl of amplified reaction mixture was analyzed by agarose (2 % w/v) gel-electrophoresis in TAE buffer (40 mM Tris, 1mM EDTA, pH -8.0). After run at 50 V for 3 h, the gel was stained with ethidium bromide (0.5 µg /ml) and photograph was taken in Gel-documentation system (Bio-Rad Laboratories, Hercules, CA, USA).

16S rRNA gene sequencing

The 16S rDNA insert was sequenced by the dideoxy- chain termination method using an automated DNA sequencer (ABI Prism; Model 3100). The 16S rDNA sequence was compared with the GenBank database by using the algorithm BLASTN programme [29] (Altschul *et al.*, 1997) to identify the most similar 16S rDNA.

Statistical analysis

Values were expressed as means of ± SD for triplicate samples. Data were analyzed by analysis of variance or by regression analysis. Differences were considered to be significant at the $P<0.05$ level.

RESULTS AND DISCUSSIONS

Effect of carbon sources on the IAA and siderophore production

Strains MR-AI and WR-W2 demonstrated diverse levels of IAA and siderophore activity in the presence of carbon source. Strain WR-W2 was proved to be the most efficient strain considering its capability to produce IAA and siderophore, utilizing a wide range of carbon source. Malate and acetate were the best sole carbon source for IAA and siderophore production by the strain MR-AI and WR-W2 as represented in Figures 1 and 2. On the other hand in presence of glucose, maltose, sucrose and fructose more or less similar amount of IAA and siderophore level was observed. Dextrose and sorbitol were identified as poor carbon source for IAA and siderophore production by strain MR-AI and WR-W2. No significant IAA was observed in the level of IAA without tryptophan (reference control). The results are similar to previous studies demonstrated that malate and acetate itself may act as siderophore, when used as a sole carbon source for bacteria [4]. Results are similar to previous report shown in Gram-negative PGPR [35, 36]. Similarly, malate acts as the most suitable carbon source for IAA bioproduction as shown in *Rhizobium* sp [37].

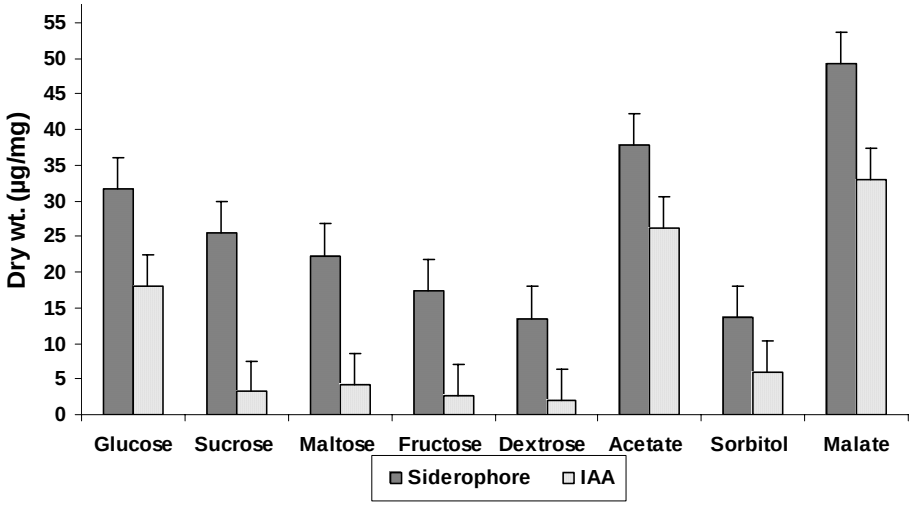

Figure-1. Estimation of IAA and siderophore production by WR-W2, in JNFb$^-$ and iron free M9 medium, respectively*.

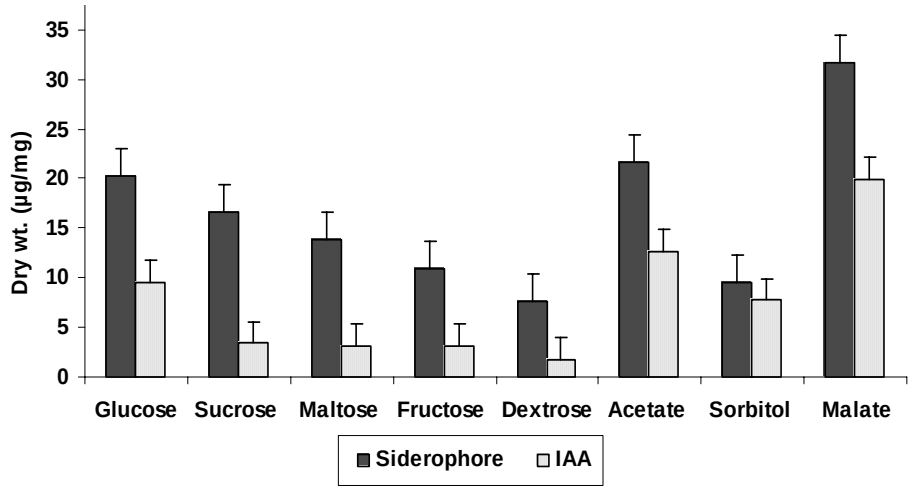

Figure-2. Estimation of IAA and siderophore production by MR-AI in JNFb and iron free M9 medium, respectively*.

*Quantitative estimations were performed after 96h of incubation at 30±2^{0}C. Where, malate and glucose was replaced with sucrose (0.5%), maltose (0.5%), fructose (1.0%), dextrose (0.5%) and sorbitol (1.0%). Culture tube containing glucose (0.5%), and malate (0.5%) was treated as control for siderophore and IAA production, respectively.

Time course study of IAA and siderophore production

To further examine production of IAA and siderophore by the strain MR-AI and WR-W2, time course study was performed at variable time period. The highest accumulation of IAA and siderophore level was observed after 96h of growth by both the strain as shown in Figures 3 and 4. Strain MR-AI and WR-W2 demonstrated a significant decline in IAA and siderophore level after 120h of growth (data not shown). Biosynthesis of IAA and siderophore was observed with the beginning of exponential phase of the growth. However, overall trend of IAA and siderophore production level during time course study are similar in both the isolates.

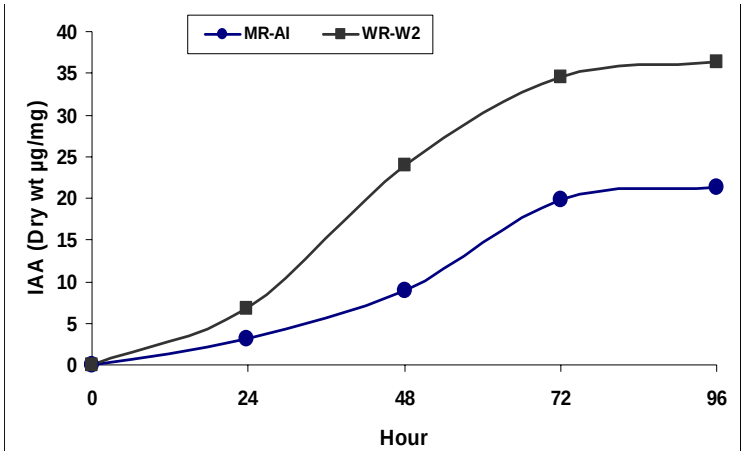

Figure-3. Time course study of IAA production by the strain MR-AI and WR-W2. Isolates were grown in JNFb$^-$ medium containing L-tryptophan 100µg/ml.

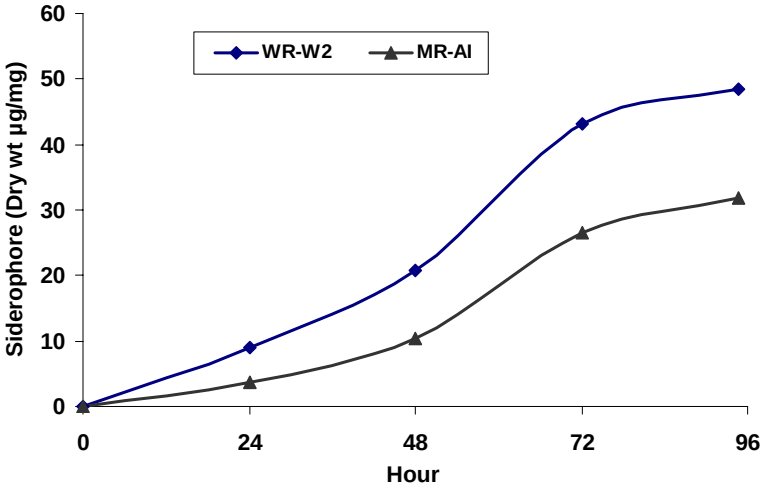

Figure-4. Time course study of siderophore production by the strain MR-AI and WR-W2. Isolates were grown in iron free M9 medium.

Simultaneous production of siderophore and IAA *in vitro*

To further determine simultaneous production of IAA and siderophore by the strain MR-AI and WR-W2 under in vitro condition. Significant amount of IAA and siderophore was observed by both the strain (Figure-5). However, the level of IAA and siderophore was inhibited as compared to control by both the strains. Strain WR-W2 was found to be more efficient in terms of IAA and siderophore production together as compared to MR-AI. To our knowledge this is the first report demonstrating IAA and siderophore production activity, together under in vitro conditions by the isolates MR-AI and WR-W2. However, in control test tube IAA and siderophore production was not observed which was without tryptophan and 1 µM $FeSO_4.7H_2O$ added culture flask, respectively. Estimation of IAA and siderophore in culture filtrate at different time intervals shows a non-linear, time dependent accumulation of IAA, which is similar to previous report [38]. Moreover, in response to iron deprivation, *E. coli* and *B. subtilis* both produce catecholic siderophore, which are very similar in structure [17, 39]. Dihydroxybenzoic acid (DHBA) is produced constitutively by all the siderophore-producing bacteria and is thus not considered a true siderophore.

Effect of Fe-EDTA on siderophore production

To further examine efficiency of halo zone formation capability of the strain MR-AI and WR-W2 were grown on iron free M9 agar medium, in presence of containing Fe-EDTA 0, 10 and 20 µM. (Figure-6). The effect of chelation on siderophore production demonstrated maximal increase of siderophore halo zone diameter, in control (0 µM.). Further increase in Fe-EDTA caused a linear decrease to the siderophore production. Colony diameter and halo zone diameter of the strain MR-

AI and WR-W2 were recorded after 96h of growth (Table-1). The result suggest that the bacterial strain WR-W2 showed more efficient halo zone forming strain as compared to MR-AI. At 20 µM Fe-EDTA siderophore production was found inhibited (Figure-6).

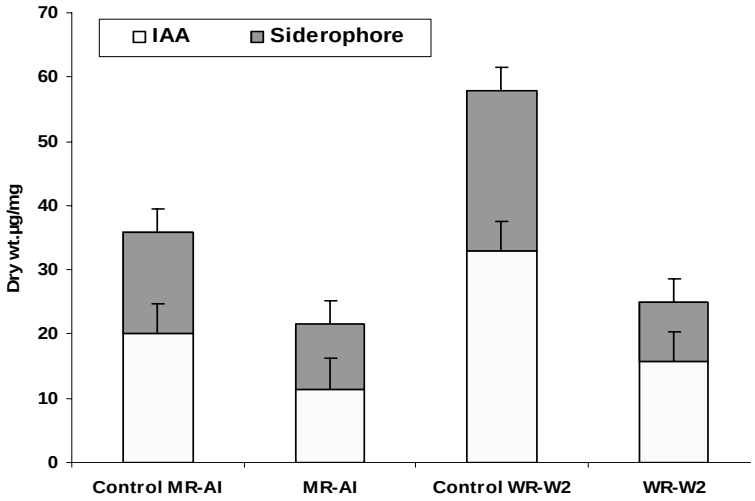

Figure-5. Simultaneous production of IAA and siderophore by MR-AI and WR-W2. Isolates were grown in iron free M9 medium, exogenously supplemented with L-tryptophan 100 µg/ml. Quantitative estimations was performed after 96h of growth at 30±2^{0}C. Data's presented are means ± S.D for triplicate samples under identical conditions, variation (S.D) are within ± 0.05.

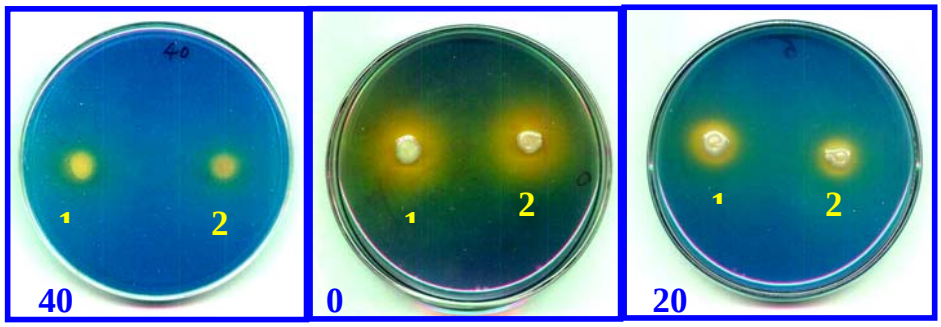

Figure-6. Qualitative assessment of halo zone production onto CAS added M9 medium in presence of Fe-EDTA (0.0, 20.0 and 40.0 µg/ml) by the strain MR-AI and WR-W2. Where 1: MR-AI and 2: WR-W2.

Table-1. Measurement of CD and HZD in presence of Fe-EDTA[#].

Isolates	Fe-EDTA					
	(0 µg/ml)		(20 µg/ml)		(40 µg/ml)	
	CD	HZD	CD	HZD	CD	HZD
WR-W2	0.60 ± 0.2	2.40 ± 0.2	0.50 ± 0.2	1.40 ± 0.2	0.20 ± 0.1	0.60 ± 0.1
MR-AI	0.70 ± 0.1	2.20 ± 0.1	0.40 ± 0.3	1.10 ± 0.2	0.20± 0.2	0.55 ± 0.2

Measurement of colony diameter (CD) and halo zone diameter (HZD) was after 96h of growth at 30±2^{0}C in presence of Fe-EDTA (0.0, 20.0 and 40.0 µg/ml) by the strain MR-AI and WR-W2. Where, the strains grown without Fe-EDTA were treated as control, which was amended with 1 µM of $FeSO_4.7H_2O$. Diameters are represented in centimeter (cm).

Effect of $FeCl_3.6H_2O$ on siderophore production

To further investigate the effect of $FeCl_3.6H_2O$ (0, 5, 10, 15 and 20 µM) on the siderophore production, strain MR-AI and WR-W2 was grown in M9 broth medium. The result suggest a linear increase in siderophore production as the concentration of $FeCl_3.6H_2O$ was increased from 0 to 15 µM, However, at 20 µM the siderophore level was found inhibited (Figure-8). Effect of Fe^{3+} on siderophore production has been well studied [40]. Reports suggest inhibition of siderophore production at 10 µM Fe^{3+} concentration [41]. In contrast, siderophore production was demonstrated at 50 µM Fe^{3+} [42]. In present study, the highest amount of siderophore production was evident at 20 µM Fe^{3+}.

TLC of IAA and siderophore production

To further check IAA and siderophore production by the strain MR-AI and WR-W2, TLC was performed (Data not shown). IAA was identified by spraying the plates with Ehmanns reagent [30]. Development of single pink spot indicated presence of siderophore after spray CAS containing indicator reagent [22]. Spots with Rf value were identical to the authentic standard 2, 3 DHBA sample, which, were having Rf value 0.90 for siderophore and IAA test.

Characterization of siderophores

To further characterize the siderophore production by the strain MR-AI and WR-W2, were grown in iron free M9 medium. Qualitative identification demonstrates release of catecholate type of siderophore production by both the strain. Pink color was observed in both the strain after the addition of Arnow`s reagent. Simultaneously, quantitative estimation was also preformed which indicated the level of catecholate was higher in strain WR-W2 as compared to MR-AI (Figure-7). However, in control tube development of any color was not observed.

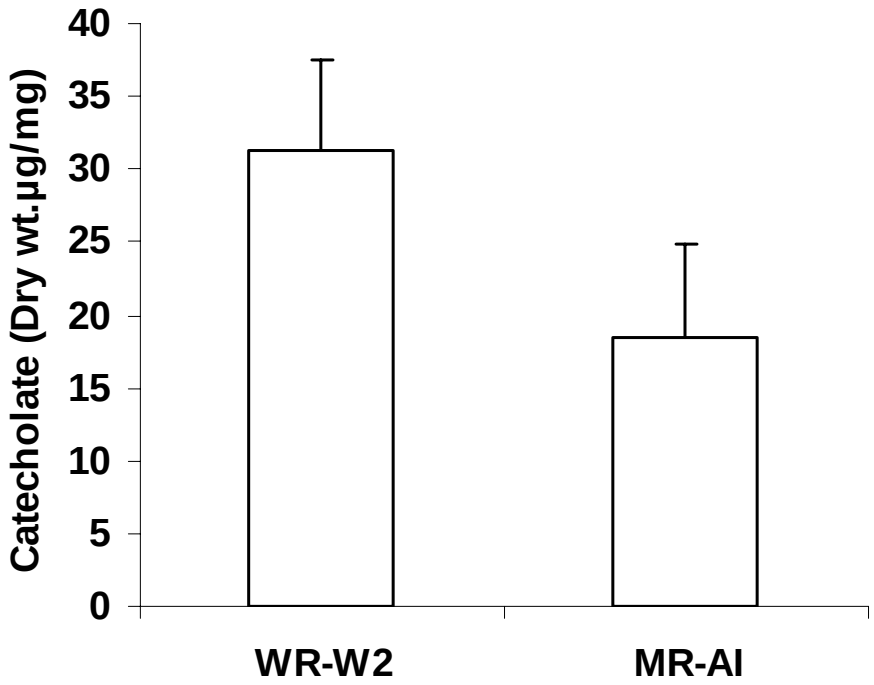

Figure-7. Production of catecholate type of siderophore. Isolates were grown in iron free M9 medium and estimation was performed after 24h of growth at 30±2^{0}C.

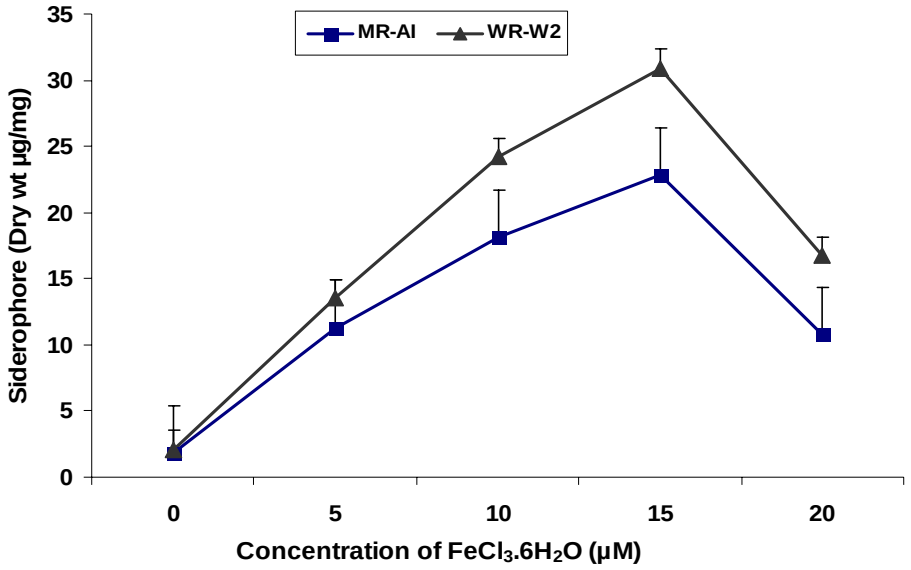

Figure-8. Siderophore production in presence of 0, 5, 10, 15 and 20 µM of $FeCl_3$ $6H_2O$. Quantitative estimation was performed after 96h of incubation at 30±2^{0}C.

Effect of strains MR-AI and WR-W2 on rice plant growth

To determine the effect of the MR-AI and WR-W2 on growth of rice variety *Pusa sugandha III*, seeds were germinated on FC^-N^- semisolid medium in presence and absence of tryptophan (100µg/ml). Fresh root/shoot length, root/shoot wt. was recorded after one week of growth at 30±2^{0}C. Simultaneously chlorophyll a was also estimated. Data presented in Tables 2 and 3 show diverse level of plant growth promoting ability by the two strains. Without tryptophan, the root elongation, number of rootlets of germinating seeds was highest with both the strains as compared to the control, whereas, the shoot length was increased in MR-AI and WR-W2 strains. The pot culture experiment clearly demonstrate the beneficial effects of bioinoculant strain MR-AI and WR-W2 with significant increase ($P<0.05$) in the plant growth attributes in untreated control medium. The stimulating effect of plant growth was even higher when growing bacterial cells in appropriate concentration (2×10^5 cfu^{-1}) resulted in significant $P<0.01$ reduction of plant fresh weight. More over this ability is dependent on the presence of tryptophan which is one of the main compounds present in several plant exudates [31]. Plant-growth-promoting activity of *B. amyloliquefaciens* FZB42 and other strains belonging to the *B. subtilis/ B. amyloliquefaciens* group are well documented [32, 33, 34]. Moreover, production of IAA by *B. amyloliquefaciens* and their PGP effects on crop seedling has been reported [12, 33, 6].

DNA sequencing

The 16S rRNA gene sequences were submitted to the National Center for Biotechnology Information database (NCBI) on http://www.ncbi.nlm.nih.gov. The 16S rDNA sequence of strain WR-W2 (*B. subtilis*) and strain MR-AI (*B.amyloliquefaciens*) has been deposited in the GeneBank database under accession number (EU327503 for 400 bp of V2-V3 region and [FJ222553] for 1.5 Kb and ([EU327505] for 400 bp of V2-V3 region and [FJ222551] for 1.5 Kb), respectively. Altogether, the strains MR-AI and WR-W2 could serve as an excellent model to study the physiological and biochemical mechanism of IAA and siderophore production and provide tremendous opportunities in environmentally sustainable approach to increase crop production.

Table-2. Growth promotion activity of WR-W2 and MR-AI on *Pusa sugandha III* in presence of tryptophan (100µg/ml)*.

Isolates	**Length** (cm)		**Fresh wt.** (mg)		**Chlorophyll a** (µg gm fresh wt^{-1})
	Shoot	**Root**	**Shoot**	**Root**	
MR-AI	11.7 ± 2	6.1 ± 3	33.8 ± 3	20 ± 2	8.8 ± 2
WR-W2	16.9 ± 3	9.3 ± 3	41.5 ± 3	23.5 ± 3	14.8 ± 3
Uninoculated*	9.11 ± 3	3.3 ± 3	23.2 ± 3	13 ± 2	4.8 ± 2

Table-3. Growth promotion activity of WR-W2 and MR-AI on *Pusa sugandha III***.

Isolates	**Length** (cm)		**Fresh wt.** (mg)		**Chlorophyll a** (µg gm fresh wt^{-1})
	Shoot	Root	Shoot	Root	
MR-AI	12.9 ± 2	6.23 ± 3	40 ± 3	56 ± 3	10.6 ± 3
WR-W2	9.73 ± 2	12.16 ±3	29 ± 3	34 ± 3	9.4 ± 3
Control*	5.12 ± 3	3.3 ±3	10.9 ± 3	16.3 ± 3	5.3 ± 3

Results are based on average of three experiments conducted separately under identical conditions. Two-days-old germinated seeds of *Pusa Sugandha III* were planted in culture tubes for bacterization experiments. 2 x 10^8 CFU ml^{-1} of bacterial culture was inoculated in each tube. The presented data are based on observation made after 7 day of inoculation. *Where control was added with tryptophan (100µg/ml) but uninoculated. ** The reference control was uninoculated.

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