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Biodegradation of isoproturon using a novel *Pseudomonas aeruginosa* strain JS-11 as a multi-functional bioinoculant of environmental significance

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ABSTRACT

Biodegradation of phenylurea herbicide isoproturon was studied in soil microcosm bioaugmented with a novel bacterial strain JS-11 isolated from wheat rhizosphere. The molecular characterization based on 16SrDNA sequence homology confirmed its identity as *Pseudomonas aeruginosa* strain JS-11. The herbicide was completely degraded within 20 days at ambient temperature with the rate constant of 0.08 day^{-1}, following the first-order rate kinetics. In stationary phase, at a cell density of 6.5×10^9 CFU mL^{-1}, the bacteria produced substantially increased amounts of indole acetic acid (IAA) in the presence of tryptophan as compared with the control. Also, the bacteria exhibited a time-dependent increase in the amount of tri-calcium phosphate solubilization in Pikovskaya's medium. Further screening of the strain JS-11 for auxiliary activities revealed its remarkable capability of producing the siderophores and hydrogen cyanide (HCN), besides antifungal activity against a common phytopathogen *Fusarium oxysporum*. Thus, the versatile *P. aeruginosa* strain JS-11 with innate potential for multifarious biological activities is envisaged as a super-bioinoculant for exploitation in the integrated bioremediation, plant growth and disease management (IBPDM) in contaminated agricultural soils.

1. Introduction

The agrochemical isoproturon (N,N-dimethyl-N′-[4-(1-methylethyl)phenyl]urea) is a systemic phenylurea herbicide, which is mainly used for pre- and post-emergence control of annual grasses and broad-leaf weeds in cereals [1]. Due to its intensive and repeated usage as well as its persistence in the environment, it is frequently detected in the surface and groundwater bodies at concentrations exceeding the European Union drinking water limit of 0.1 $\mu g\,L^{-1}$ [2]. It is reported to be directly or indirectly toxic to a wide variety of organisms including aquatic invertebrates [3], fresh water algae [4], microbial communities [5], plants [6], and is also reported to be carcinogenic to animals and humans [7,8]. Therefore, understanding the fate of isoproturon in soil is important for developing strategies to minimize its environmental impact. Indeed, microbial degradation is the primary mechanism for mineralization of isoproturon [9]. Several bacterial strains such as *Sphingomonas* sp. SRS2 [10], *Arthrobacter* sp. N2 [11], *Sphingomonas* sp. F35 [12], *Methylopila* sp. TES [13], and *Sphingobium* strains [14] have been reported to degrade this herbicide. Sørensen et al. [15] have demonstrated the enhancement of isoproturon degrading capability of *Sphingomonas* sp. SRS2, when grown in co-culture with strain SRS1, not able to degrade isoproturon. Similarly, several bacterial consortia have been reportedly involved in the degradation of pesticides [16], however, the studies related to isoproturon degradation using pure bacterial isolates from agricultural soil are limited. It has also been observed that one or more previous applications of the same pesticide or another pesticide with a similar chemical structure result in its increased rate of microbial degradation in soil. Even some of them failed to provide adequate control against their target pests and pathogens [17]. This accelerated or enhanced degradation may cause economic losses to farmers as it forces them to increase the pesticide use by several-fold with only a nominal increase in crop output [17]. Furthermore, the indiscriminate use of pesticides for a longer time may also pose another serious problem of pesticide residue accumulation in soil, which could be detrimental to both the soil and human health, and demand effective remedies. Therefore, in order to overcome these problems, a more functional and eco-friendly approach involving microbes with multi-functional activities is essentially needed in order to compensate the loss of biocontrol activity due to accelerated pesticide degradation in contaminated soils.

As a prominent group of rhizospheric microorganisms, the pseudomonads have substantially contributed to bioremediation, plant growth promotion and biocontrol of pathogens. Members of

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this versatile genus have demonstrated a catabolic potential with inherent capacity for xenobiotics degradation [18]. Mechanisms that make pseudomonads an effective biocontrol agent against pathogens involve the production of siderophores [19], antibiotics [20], and extracellular enzymes [21]. Therefore, the use of such rhizospheric bacteria in bioremediation of pollutants in soils has been proposed for disseminating the desired bioinoculants in contaminated soils. To the best of our understanding, very few reports are available on the microorganisms with multifaceted characteristics that confer concurrent plant growth promoting and biocontrol abilities besides their role in pesticide degradation [22,23]. Thus, the prime objective of the present study was to screen the isoproturon resistant bacterial isolates from wheat rhizospheric soil for selection of an efficient strain with inherent isoproturon catabolizing ability, and multiple auxiliary plant beneficial traits. The most promising isoproturon degrader strain JS-11 has exhibited inorganic phosphate solubilizing activity, indole acetic acid (IAA), siderophores and hydrogen cyanide production in addition to antifungal activity. This novel bacteria could be exploited as a natural super-bioinoculant for providing an effective and eco-friendly alternative solution to the herbicide contamination, plant growth and pathogen control problems, based on its innate bioremediation, plant growth and disease management (IBPDM) potential.

2. Materials and methods

2.1. Chemicals and reagents

Technical grade (99% purity) isoproturon (CAS No. 34123-59-6) was obtained from Sigma Chemical Company, St. Louis, MO, USA. Isoproturon (75% WP) was obtained from Modern Insecticide Ltd., India. All other chemicals, solvents and nutrient media were purchased from Sigma Chemical Company (USA), Sisco Research Laboratory (India) and Hi Media (India), respectively.

2.2. Isolation and characterization of IPU degrading bacteria

Bacteria capable of degrading isoproturon were isolated from wheat rhizosphere of isoproturon contaminated soil by the enrichment culture technique. The soil homogenate was inoculated in 50 mL nutrient broth (yeast extract, 1.5; beef extract, 1.5; peptone, 5.0; sodium chloride, 5.0; g L^{-1}, pH 7.0 ± 0.2) supplemented with 100 μg mL^{-1} isoproturon and incubated at 28 ± 2 °C. Subsequently, 0.2 mL of this culture was plated on M9 mineral salt agar (ammonium sulphate, 1.0; dipotassium hydrogen orthophosphate, 1.0; disodium hydrogen orthophosphate, 2.1; magnesium sulphate, 0.01; calcium chloride, 0.10; ferric chloride, 0.001; copper sulphate, 0.040; sodium molybdate, 0.002; g L^{-1}, pH 7.2 ± 0.2) supplemented with 200 μg mL^{-1} of isoproturon. The resulting colonies were repeatedly sub-cultured in M9 medium containing 500 μg mL^{-1} of isoproturon to confirm their catabolizing ability. A total of 185 colonies were picked up from M9 agar plates and the isolates were initially screened for isoproturon tolerance up to 3 mg mL^{-1} using minimum inhibitory concentration (MIC) technique. Identification and characterization of the isolate JS-11 was done on the basis of the colony morphology, biochemical characteristics following Bergey's manual of systematic bacteriology [24]. The catalase and oxidase tests were performed using standard procedures. In brief, nutrient agar slants inoculated with strain JS-11 were incubated at 28 °C for 48 h. Appearance of O_2 bubbles upon addition of 3% H_2O_2 was considered as positive test for catalase. The oxidase activity was carried out using disc assay (Hi-Media, India), where the change in color of disc to deep purple was regarded as a positive test. The nitrate reduction was performed by the traditional colorimetric procedure [25]. Nitrate reduction was observed in the culture medium supplemented with nitrate of sulfanilic acid and N,N-dimethyl-1-naphthylamine, which react with nitrite to produce a pink/red coloration indicative of nitrate reduction. Also, the metabolic profile of the strain including the carbohydrate and amino acid utilization was determined using BIOLOG GN plates following the recommendations of the producer (Biolog Inc., Hayward, CA, USA). The phylogenetic analysis was based on 16SrDNA sequence homology [26].

2.3. PCR amplification, cloning and sequencing of bacterial 16SrDNA

The isolate JS-11 was grown in nutrient broth at 28 ± 2 °C. Cells were harvested after 24 h and processed immediately for DNA isolation by standard procedure [27]. Using the purified genomic DNA as target, the gene coding for 16SrRNA was amplified employing primers, fD1 (5′-AGAGTTTGATCCTGGCTCAG-3′) and rD1 (5′-AAGGAGGTGATCCAG CC-3′) [28] complementary to the 5′ and 3′ regions of eubacterial 16SrRNA genes, respectively. The PCR amplification was carried out in a final volume of 50 μL. Briefly, the amplification reaction containing 50 ng template DNA, 30 pmole each of universal primers primer, 0.2 mM dNTPs, and 2.5 U Taq polymerase (Gibco-BRL, USA) in 1 μL PCR buffer (Sigma, USA) was run on an Biometra personal cycler. The amplification reaction was cycled as follows: 94 °C for 1 min, 55 °C for 1 min; 72 °C for 1 min and a post-dwell at 72 °C for 3 min for 30 cycles. The amplicon was analyzed on 1.5% agarose gel in 1× TAE, run at 50 V for 2 h and purified using QIA quick PCR purification kit, Qiagen, USA. The purified amplicon (1.5 kb) was sub-cloned with in *lac*Z gene in pGEM-T easy vector (3.015 kb) (Promega, USA). The selected clone was subjected to sequencing of 16SrRNA gene fragment with SP6 and T7 sequencing primers using ABI prism 3730 sequencer. The sequence of JS-11 was submitted to GenBank NCBI database and accession no. EF378653 obtained. The homologous 16SrRNA gene sequences were subjected to the multiple sequence alignments by ClustalW multiple alignment method using BioEdit (version 5.0.9). Phylogenetic tree was constructed by the neighbour-joining (NJ) method with nucleotide pair-wise genetic distances corrected by Kimura two-parameter method [29] using TreeCon tool. The reliability of tree topology was subjected to a bootstrap test and numbers at nodes indicate bootstrap support values as a percentage of 1000 replications.

2.4. Growth kinetics of isoproturon degrading isolates

The bacterial isolate was freshly grown in M9 medium (pH 7.2) containing 200 μg mL^{-1} isoproturon as a sole source of carbon. For determination of growth kinetics, 0.1 mL culture of the isolate JS-11 was inoculated into 20 mL each of the nutrient broth and mineral salt medium without and with 1 mg mL^{-1} isoproturon in 100 mL Erlenmeyer flask. The culture was incubated at 28 ± 2 °C in a shaker water bath. At regular time intervals, the optical density at 600 nm was measured and the viable bacterial counts in terms of colony forming units (CFU mL^{-1}) were determined by plating the serially diluted cultures on agar plates. The growth curves were obtained by plotting the log CFU mL^{-1} as a function of time.

2.5. Kinetics of biodegradation

The biodegradation of isoproturon in soil microcosm was performed as described earlier [22,23,30]. The soil (sandy loam) microcosms in triplicate consists of 1 kg each of sterilized rhizospheric soil (control), unsterilized rhizospheric soil, and bioaugmented unsterilized rhizospheric soil were prepared in autoclaved earthen pots. The soil samples were amended with isoproturon (EC 75%) at a loading of 15 kg a.i. ha^{-1} (10× of recommended dose) equivalent to a.i. 10 mg kg^{-1} of soil. The

bioaugmented soil samples were enriched with freshly grown culture of isolate JS-11 at a cell density of 10^7 CFU g^{-1} soil. At different time points from 0 to 20 days, aliquots of 5 g soil in triplicate (total 15 g soil) were removed from the microcosms. The control consisting of sterilized soil with same amount of isoproturon was run simultaneously under identical conditions. The samples were subjected to organic solvent extraction three times with HPLC grade ethyl acetate. The extracts were filtered and evaporated to near dryness on a rotary thin film evaporator. The residues were dissolved in 5 mL of acetonitrile and stored at 4 °C until analysis. The HPLC analysis was performed on Waters HPLC System coupled with 2487 dual λ UV/visible detector. Separation was achieved by using a C-18 Novapak, 4 μm (Waters Corp., Milford, MA, USA) radically compressed cartridge using ethyl acetate:water in the ratio of 70:30 as a mobile phase. The flow rate was 1 mL min^{-1} and absorbance was read at 240 nm. The degradation rate constant (k) was determined using the algorithm $C_t/C_0 = e^{-kt}$ where C_0 is the amount of isoproturon in the soil at time 0, C_t is the amount of isoproturon in soil at time t and, k and t are the rate constant (day^{-1}) and degradation period in days, respectively.

2.6. Assessment of characteristics for plant growth promotion

The production of indole acetic acid (IAA) and inorganic phosphate solubilization were determined as an index of plant growth promoting ability of the strain JS-11. IAA was quantitated according to the method of Bric et al. [31] in JS-11 inoculated nutrient broth without and with tryptophan (250 and 500 μg mL^{-1}) at 28 ± 2 °C. For IAA inhibition assay, isoproturon in the concentration range of 50–500 μg mL^{-1} was added to the tubes containing JS-11 in nutrient broth supplemented with tryptophan (500 μg mL^{-1}) and processed for IAA measurements on day 4, following the standard procedure. Also, the phosphate solubilizing activity of the strain JS-11 was determined by measuring the zone size formed by solubilization of insoluble phosphate on Pikovskaya's agar plates. Furthermore, the soluble phosphate released was quantitated following the method of King [32]. For determining the herbicide mediated inhibition of inorganic phosphate solubilization, the isoproturon in concentration range of 50–500 μg mL^{-1} was added to the tubes containing JS-11 in Pikovskaya's liquid medium and processed after 5 days of incubation for phosphate measurements, following the standard method as referenced above.

2.7. Assessment of characteristics for biocontrol activity

The siderophore and hydrogen cyanide (HCN) production as well as the anti-fungal activity were determined as potential biocontrol attributes of strain JS-11. Siderophore production was assessed by chrome azurole S (CAS) assay [33]. The fluorescent pigment in culture supernatant was determined by UV spectral analysis with characteristic peak of pyoverdine (Pvd) siderophore between 402 and 408 nm [34]. All experiments were performed in deferrated media and glassware. The production of HCN was observed according to the method of Lorck [35] in presence and absence of 75 μg mL^{-1} $FeCl_3$. The antagonistic ability of the isolate was tested against the *Fusarium oxysporum* on Sabouraud dextrose agar (glucose, 40.0; peptone, 10.0; agar–agar, 15.0 g L^{-1}, pH 7.0 ± 0.2) by agar diffusion and dual culture assays.

3. Results and discussion

3.1. Isolation and characterization of isoproturon degrader strain JS-11

Using enrichment culture technique, the most efficient isoproturon degrader isolate JS-11was screened out of a total of 185 isolates, assessed for isoproturon tolerance in the range of 100 μg mL^{-1} to 3 mg mL^{-1} isoproturon on M9 agar plates. Amongst all, the isolate JS-11 exhibited maximum isoproturon tolerance at the highest concentration of 2.5 mg mL^{-1} in minimum inhibitory concentration (MIC) studies, and has exhibited effective utilization of isoproturon as a sole source of carbon in M9 liquid medium (data not shown). The growth kinetics studies in M9 medium containing 1.0 mg mL^{-1} isoproturon exhibited a prolonged (7 h) lag phase as an adaptation period before the exponential growth resumed as compared to much faster growth in nutrient broth (Fig. 1). The kinetic data revealed the growth rate (n) and generation time (t) of the strain JS-11 as 0.21 h^{-1} and 4.6 h in mineral salt medium and 0.61 h^{-1} and 1.6 h in rich medium. The isolate was found to be aerobic, non-spore forming, Gram-negative, rod shaped bacteria producing small, circular colonies on the nutrient agar plates. The freshly grown culture showed positive tests for oxidase and catalase, and exhibited the ability of nitrate reduction, glucose fermentation, and amino acid utilization and, therefore, presumptively identified as *Pseudomonas aeruginosa* sp. (Table 1). Molecular characterization based on 16SrDNA homology of a partial sequence (1191 bp) with the sequences in NCBI database confirmed the identity of strain as *P. aeruginosa* strain JS-11. Multiple alignments and phylogenetic analysis (Fig. 2) revealed that the strain JS-11 (EF378653) cluster with *Pseudomonas* spp. (DQ112026 and DQ166807) exhibiting close relationship with 100% similarity.

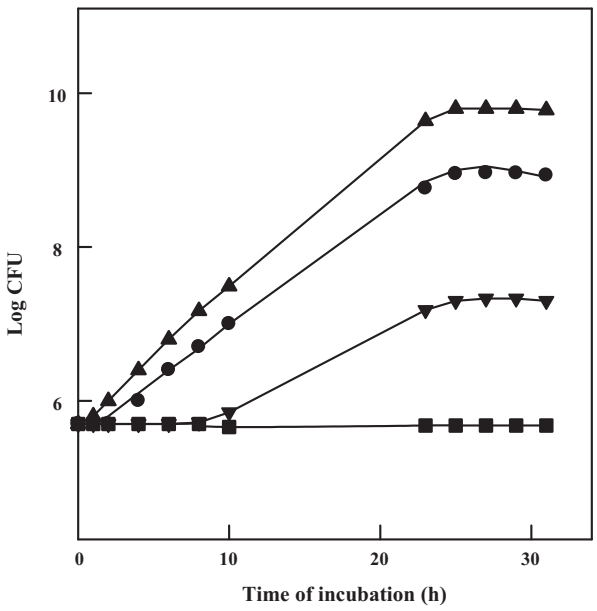

Fig. 1. Growth pattern of bacterial strain JS-11 under different growth conditions. The curves represented are in: (■) M9 medium and (▲) nutrient broth, alone; (▼) and (●) M9 medium and nutrient broth supplemented with 1000 μg mL^{-1} isoproturon, respectively.

3.2. Kinetics of isoproturon biodegradation

The degradation isotherms exhibiting complete disappearance of isoproturon within 20 days with the rate constant of 0.08 day^{-1}, following the first-order rate kinetics are presented in Fig. 3. The disappearance of isoproturon in unsterilized soil occurred due to the presence of indigenous heterotrophic microflora in rhizospheric soil. Moreover, the addition of strain JS-11 has significantly enhanced the degradation to the extent of 40% in bioaugmented soil after 20 days of incubation under identical conditions. However, the control sterilized soil containing the same amount of isoproturon does not exhibit any degradation. The microbial population size in microcosms varies in the order of inoculated rhi-

Table 1
Morphological and biochemical characteristics of bacterial strain JS-11.

Characteristics	JS-11	PAO1
Colony morphology	Large, irregular, greenish yellow,	Greenish yellow
Gram stain	−ve Bacilli	−ve Bacilli
Biochemical test		
Indole test	−	−
Voges-Proskauer	−	−
Methyl red	−	−
Catalase	+	+
Oxidase	+	+
Nitrate reduction	+	+
Sugar fermentation		
Sucrose fermentation	−	−
N-Acetyl-D-glucosamine	+	+
D-Fructose	−	−
D-Glucose	+	+
Mannose	+	+
D-Sorbitol	−	−
D-Trehalose	−	−
Organic acid utilization		
Succinic acid	+	+
Acetic acid	+	+
α-Hydroxybutyric acid	−	−
Lactic acid	+	+
Amino acid utilization		
Aspartic acid	+	+
Glutamic acid	+	+
D-Alanine	+	+
L-Alanine	+	+
Glycil-L-butyric acid	+	+
L-Histidine	+	+
Hydroxy-L-proline	+	+
L-Leucine	+	+
L-Serine	+	+
Presumptive identification	*Pseudomonas aeruginosa*	

Data for *Pseudomonas aeruginosa* sp. nov. strain PAO1 were taken from Ferguson et al. [52] for comparative analysis of our strain JS-11.Characterstics are scored as: (+) utilized; (−) not utilized.

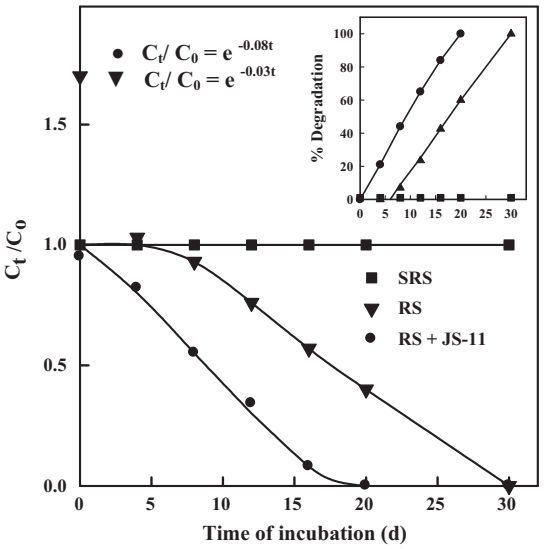

Fig. 3. Rate of disappearance of isoproturon in soil microcosm augmented with an axenic culture of isoproturon degrading isolates. The inset shows the percentage degradation of isoproturon as a function of time. SRS: sterilized rhizosphere soil; RS: rhizosphere soil; RS+JS-11: inoculated rhizosphere soil.

zosphere > unsterilized rhizosphere > sterilized rhizosphere soils, which corroborates well with the earlier studies [36] and suggest that the degradation rates are associated to microbial biomass. A time-dependent reduction in the parent compound with appearance of multiple secondary peaks in HPLC profiles (data not shown) affirmed the microbial transformation of isoproturon in soil. Since, the identification of isoproturon metabolites have been extensively studied earlier [9,37,38], the metabolite characterization is beyond the scope of this study. However, it is imperative to realize the importance of identifying the degradation products to assess the impact of any polluting agent as it may undergo transformation yielding compounds more toxic than the parent compound. Tixier et al. [11] identified substituted aniline as a sole metabolite

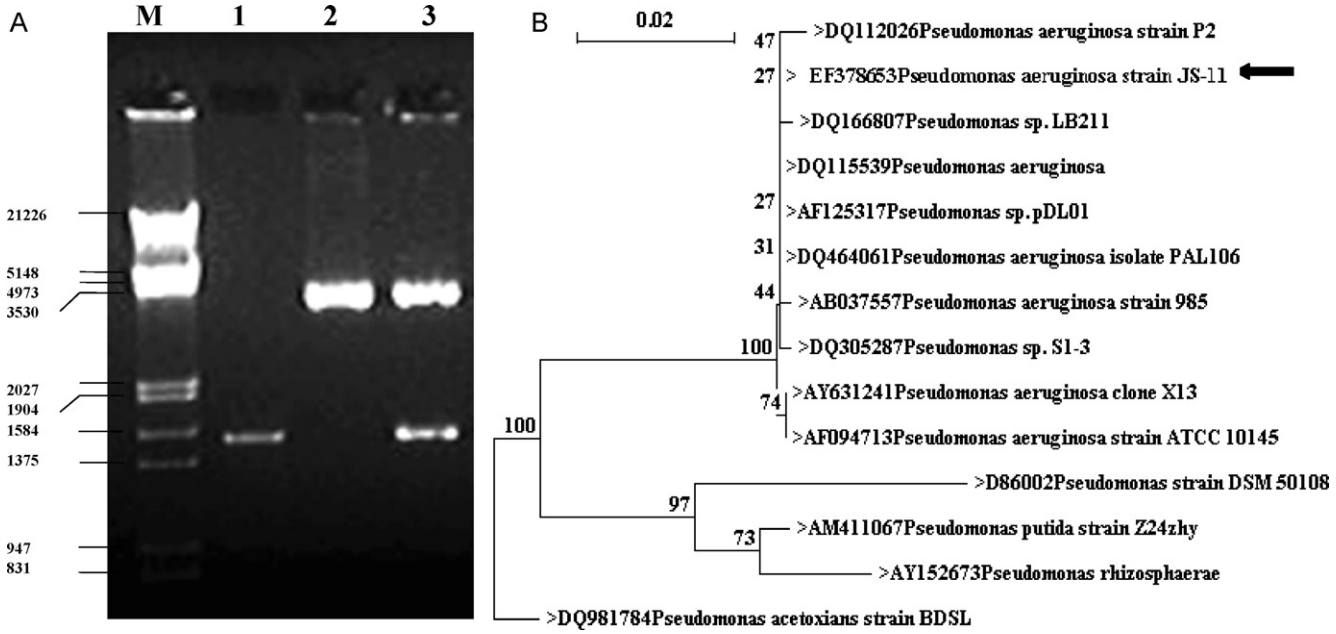

Fig. 2. Panel-A represents the agarose gel showing the bands of 16SrRNA amplicon and pDrive cloning vector with insert. Lanes are indicated as-M: marker (lambda DNA *Hind* III/*Eco*R 1 digest); lane 1: PCR amplicon of JS-11 16SrRNA gene; lane 2: *Eco*R I digested control plasmid vector; lane 3: *Eco*R I digested recombinant plasmid. Panel-B shows the phylogenetic relationship based on 16SrRNA gene nucleotide sequences between the *Pseudomonas aeruginosa* strain JS-11 and reference sequences retrieved from NCBI GenBank. The scale bar indicates the numbers of nucleotide substitutions per site.

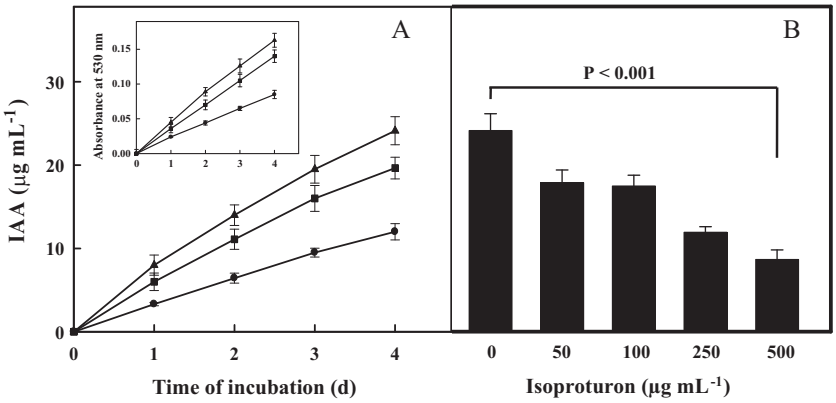

Fig. 4. Assessment of indole acetic acid production by the strain JS-11. Panel-A shows the amount of IAA produced by strain JS-11 in nutrient broth without tryptophan (●) and supplemented with tryptophan 250 μg mL^{-1}, (■) and 500 μg mL^{-1}, (▲). The inset represents the plot of the absorbance of pink color developed, at 530 nm as a function of time. Panel-B shows the histogram indicating isoproturon concentration dependent inhibition of IAA activity.

of isoproturon produced by soil bacterial strain *Arthrobacter* sp. N2, which was reportedly transformed into 3,4-dichloroacetanilide by other microorganisms present in soil and exhibited higher non-target toxicity. Lately, Hussain et al. [39] demonstrated the transitory accumulation of three known isoproturon metabolites 3-(4-isopropylphenyl)-1-methylurea, 3-(4-isopropylphenyl)-urea, and 4-isopropylaniline and their further degradation. Indeed, many isoproturon metabolic pathways still remain to be elucidated as different parallel degradation pathways for isoproturon are proposed by defined microorganisms in agricultural soils [40]. Nevertheless, owing to its capacity of using isoproturon as a sole source of carbon and energy, the strain JS-11 inoculation to rhizospheric soil has significantly enhanced its degradation as compared to uninoculated rhizospheric soil with almost 50% reduction in isoproturon half-life. The shorter half-life of isoproturon in inoculated rhizosphere soil reflects its faster degradation, and demonstrates the ability of the strain JS-11, as an effective bioinoculant for bioremediation of isoproturon contaminated soil.

3.3. Assessment of plant growth promoting potential of strain JS-11

Our earlier observations on multifarious role of pesticide degraders have prompted us to assess the strain JS-11 for the auxiliary biological activities [22,23,30,41]. Investigation of the parameters for plant growth promotion revealed the intrinsic ability of the strain for phytohormone production, and inorganic phosphate solubilization. The results shown in Fig. 4A clearly indicate the production of substantial amounts of IAA during growth in nutrient broth. The culture of JS-11 reaches the stationary phase at a cell density of 6.0×10^9 CFU mL^{-1} after 24 h. Prolonged incubation of culture up to 4 days does not show any significant reduction in cell viability, and a sufficiently large biomass persists in the stationary phase culture even on day 4 of incubation. The results concur with the earlier observations indicating IAA production in stationary phase of the culture [23,42] due to delayed induction of a key enzyme of IAA biosynthesis pathway [43]. Several bacteria with combined biodegradation and plant growth potential are known to secrete IAA into culture media, and have shown to stimulate plant growth [22,23,44]. The presence of tryptophan in the medium substantially enhances the IAA production. Quantitative assay revealed the production of about 19.6 and 24.1 μg mL^{-1} of IAA in presence of 250 and 500 μg mL^{-1} of tryptophan, respectively as compared to 12 μg mL^{-1} IAA in medium without tryptophan. The release of this phytohormone eventually suggests the plant growth promoting ability of the strain. However, with the addition of isoproturon in culture medium, a significant inhibition (64.1%, $p < 0.001$) of IAA activity was noticed at 500 μg mL^{-1} isoproturon vis-à-vis untreated control under *in vitro* conditions (Fig. 4B). The inhibitory effect of pesticides on IAA activity has also been demonstrated earlier [30,45,46] which support our observations. Since, the strain JS-11 can tolerate isoproturon concentration up to 2.5 mg mL^{-1}, it

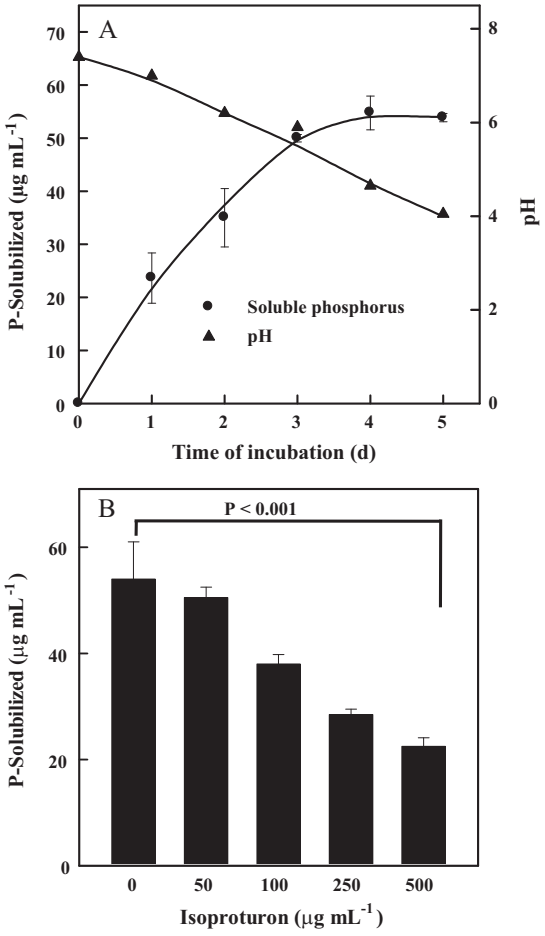

Fig. 5. Assessment of phosphate solubilizing activity of the strain JS-11 as a function of time. Panel-A shows the amount of soluble phosphate determined from the absorbance data using the calibration curve with KH_2PO_4 at 600 nm. The right-axis indicates the change in pH of the Pikovskaya's medium during growth at different time points. Panel-B shows the histogram indicating the isoproturon concentration dependent inhibition of inorganic phosphate solubilization in liquid medium.

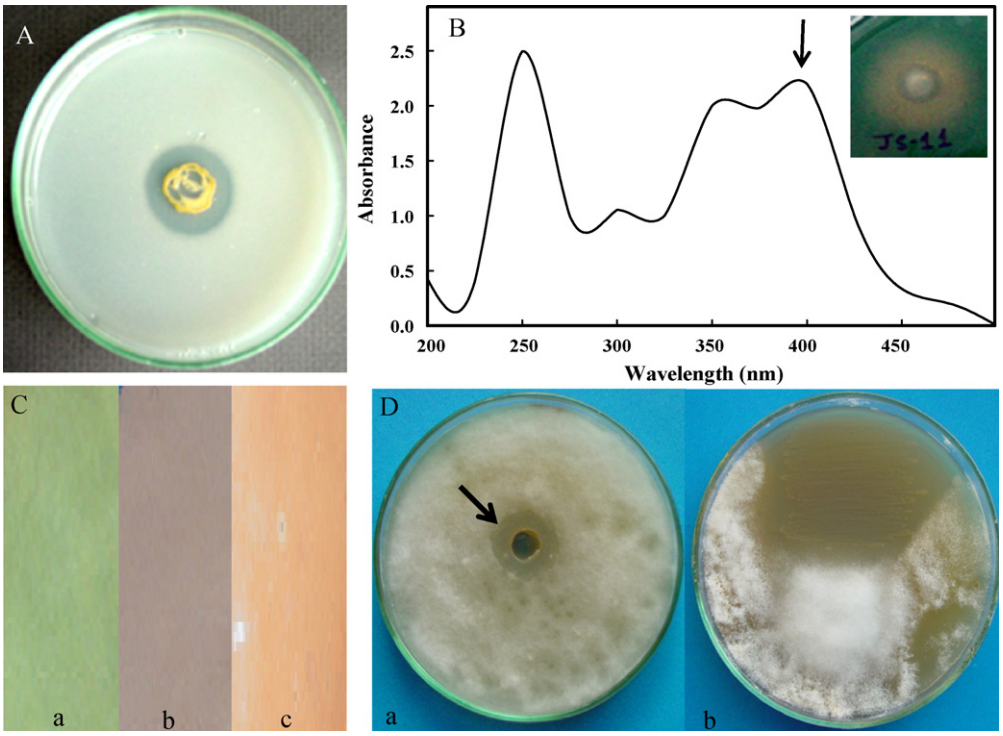

Fig. 6. Auxiliary plant growth promoting and biocontrol activities of *P. aeruginosa* strain JS-11. Panel-A reflects the zone of inorganic phosphate solubilized on Pikovskaya's agar plate. Panel-B indicates a typical peak at 405 nm characteristic of siderophore (Pvd) released from bacterial culture in King's B medium after 72 h; the inset shows the siderophore produced (red color zone) on CAS agar plate. Panel-C shows the HCN production on King's B medium, where sub-panels are (a) control (without bacteria), (b) with bacteria, (c) bacteria in presence of $FeCl_3$; panel-D shows the antagonistic ability of the strain JS-11 against *Fusarium oxysporum* on Sabouraud dextrose agar by agar diffusion and dual culture assays. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

can effectively exert the protective effect against the plant growth inhibitory action of this herbicide.

Furthermore, the growth of strain JS-11 in Pikovskaya's medium suggests the potential for phosphate solubilization (Figs. 5A and 6A). The amount of soluble phosphate released in liquid medium due to solubilization of tri-calcium phosphate has been determined to be 53.8 $\mu g\,mL^{-1}$ upon 5 days of growth (Fig. 5A). With increase in the extent of phosphate solubilization, a concomitant reduction in pH from 7.5 to 4.0 has been noticed. The relationship of decrease in pH of the culture medium with phosphate solubilization is well-established [47]. Particularly, the *Pseudomonas* spp. are well known for phosphate solubilization in soil [48]. Similar to its inhibitory effect on IAA activity, the isoproturon was also found to inhibit the phosphate solubilizing activity of the strain JS-11. An estimated 59.2% ($p < 0.001$) reduction in phosphate solubilization has been noticed at isoproturon concentration of 500 $\mu g\,mL^{-1}$ as compared to the control (Fig. 5B). These results corroborate with the observations of Madhaiyan et al. [45], who have also reported that herbicides induced inhibition of phosphate solubilization in microorganisms.

3.4. Assessment of biocontrol activity of strain JS-11

The strain JS-11 produced a reddish-brown zone on CAS agar plate due to formation of ferric–siderophore complex, indicative of the positive result for siderophore (Fig. 6B, inset). Under iron-limiting conditions, *P. aeruginosa* secretes a fluorescent siderophore called pyoverdine (Pvd), which is responsible for iron uptake [49]. Eventually the fluorescent pigment produced by strain JS-11 in King's B medium at pH 7.5 has also been identified as Pvd, on the basis the UV absorption maximum at 405 nm (Fig. 6B), which is the characteristic of Pvd-type siderophores [34]. Also, the screening of the strain JS-11 for HCN release revealed a remarkable change in color from control yellowish-green to brown in presence of iron, suggestive of HCN production. A decreased level of HCN production occurred under low iron condition as compared to HCN production in the medium containing 75 $\mu g\,mL^{-1}$ $FeCl_3$ (Fig. 6C). HCN produced by *P. aeruginosa* as a secondary metabolite, play an important role in biocontrol [50,51]. The strain JS-11 has also exhibited antagonistic activity against a phytopathogen *F. oxysporum* (Fig. 6D). Thus, the multifarious biological activities viz. biodegradation, IAA, phosphate solubilization, siderophore and HCN production, along with the antifungal property demonstrate the agronomic and environmental significance of the strain JS-11 for its plausible exploitation as super-bioinoculant.

4. Conclusions

A novel isoproturon degrading bacterium was isolated and characterized as *P. aeruginosa* strain JS-11 with the unique capability of degrading isoproturon with concurrent plant growth promoting and biocontrol potential. The ability to degrade isoproturon makes this strain a useful bioresource for remediation at the contaminated sites. Furthermore, the concurrent plant growth promoting and biocontrol abilities offer great promise for increased and sustained crop productivity even under pesticide/pathogen-induced stressed soil environment.

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