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Optimization of IAA production and P-solubilization potential in *Bacillus subtilis* KA (1) 5r isolated from the medicinal herb *Aconitum heterophyllum*-growing in western Himalaya, India

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Abstract
Bacillus species from rhizosphere are well known to promote plant growth *via* acquisition of macronutrients and production of phytohormones. The present work deals with characterization of *Bacillus subtilis* strain KA(1)5r isolated from the rhizosphere of medicinal plant *Aconitum heterophyllum* for plant growth promoting traits and optimization of IAA production and P-solubilization. Optimized cultural conditions resulted in maximum P-solubilization (197 µg/ml) after 96 h of incubation at 35 °C in PVK broth (having concentration of TCP 1%) of pH 6.0 with 1% inoculum size of culture. In case of optimized cultural conditions for IAA production, the maximum IAA production (42 µg/ml) was observed after 96h of incubation at 35 °C in LB broth (with 1.0% of tryptophan concentration) of pH 8.0 amended with an inoculum size of 2%. The selected strain *Bacillus subtilis* strain KA (1)5r could significantly enhance the growth of tomato seedlings compared with that of non- inoculated plants. From the current study, *Bacillus subtilis* strain KA(1)5r emerged as noble alternatives for IAA production and P-solubilizer, further which also resulted in root and shoot biomass generation in tomato, hence can be further used as bio-inoculants for plant growth promotion.

Keywords: *Aconitum heterophyllum*; P-solubilization; IAA production; Plant growth promotion

Introduction
The rhizosphere is the narrow zone of soil present around plant roots that support a group of metabolically versatile microorganisms (Raaijmakers *et al*., 2002) [1]. These microbes can be beneficial, deleterious and neutral to plant health (Dobbelaere *et al*., 2003) [2]. The utilization of rhizospheric microorganisms has turned into a more beneficial choice as a novel asset to be investigated for plant growth promotion. Significant number of rhizospheric bacteria have mutualistic relationship with the plant roots due to root exudates which encourages plant growth and are known as plant growth promoting rhizobacteria (PGPR). PGPR affect plant growth either directly or indirectly. Direct mechanisms involve (i) acquisition of macro or micronutrients from the environment such as nitrogen, phosphorous and iron; (ii) production or regulation of various phytohormones including auxin, cytokinin or ethylene, thereby modulating plant growth. Induced systemic resistance (ISR), antibiosis, competition for nutrients, parasitism and production of metabolites (hydrogen cyanide, siderophores) suppressive to deleterious rhizobacteria are some of the mechanisms that indirectly benefit plant growth (Bhattacharyya and Jha 2012) [3].
A number of bacterial species belonging to *Azospirillum, Alcaligenes, Arthrobacter, Acinetobacter, Bacillus, Burkholderia Bradyrhizobium, Enterobacter, Erwinia, Flavobacterium, Pseudomonas, Rhizobium* and *Serratia* have been found to be associated with rhizosphere and are able to exert beneficial effects on plant growth (Tilak *et al.,* 2005 [4]; Egamberdiyeva 2000 [5]). Among all the genera, *Bacillus* spp. are considered to be the safe microorganisms that hold remarkable abilities for synthesizing a vast array of beneficial substances (Stein, 2005) [6]. *Bacillus* spp. having potent plant growth promoting traits such as IAA production, phosphate solubilization, nitrogen fixation, and bio control attributes like production of HCN, siderophore, hydrolytic enzymes and antibiotics have been isolated from several horticultural, agricultural and medicinal crops (Mehta *et al*., 2014 [7]; Sharma *et al*., 2016 [8]; Kumar *et al*., 2015 [9]). Several other workers have also found the biocontrol activities of *Bacillus* against manycommon phytopathogens (Chung *et al*., 2008 [10], Gajbhiye et *al*., 2010 [11]). However, it is likely that the most effective PGPR strains act *via* multiple mechanisms.

Phosphorus (P), the essential macro nutrient required for biological growth and development, is copiously accessible in soils in organic as well as inorganic forms. Despite its large reservoir, the amount of available P to plants is generally low because of its presence in insoluble, non utilizable form in soil. Inorganic forms of P occur in soil mostly in insoluble mineral complexes such as apatite and organic forms including inositol phosphate, phosphomonoester and phosphotriesters (Glick, 2012) [12]. Several PGPRs have been reported to exhibit the capacity of mineralization and solubilization of inorganic as well as organic insoluble complex forms of phosphorus by secreting organic acids or extracellular hydrolytic enzymes (acid phosphatase), thereby improving the accessibility of nutrients to plants (Bhattacharya and Jha, 2012 [3]; Mehta *et al*., 2014 [7]; Sharma *et al*., 2016 [8]).

Many PGPR can produce auxins (Omer *et al*., 2004 [13]; Gupta *et al*., 2015 [14]) to exert particularly strong effects on root growth (Jha and Saraf, 2015) [15] and architecture (Vacheron *et al*., 2013) [16]. Indole-3-acetic acid (IAA) is the most widely studied auxin produced by PGPR. It is involved in plant-microbe interactions (e.g., Ahemad and Kibret, 2014[17]; Afzal *et al*., 2015 [18]). The function of exogenous IAA is dependent on the endogenous IAA levels in plants. At optimal IAA concentrations in plants, application of bacterial IAA may have neutral, positive, or negative effects on plant growth (Spaepen and Vanderleyden, 2011) [19]. PGPR that produce auxins have been shown to elicit transcriptional changes in hormone, defense-related, and cell wall related genes, induce longer roots (Hong *et al*., 1991) [20], increase root biomass and decrease stomata size and density (Llorente *et al*., 2016) [21], and activate auxin response genes that enhance plant growth (Ruzzi and Aroca, 2015) [22]. Since IAA has been found to be very important for plant growth and development, extensive studies have been performed on IAA after its discovery as a plant hormone. It has been found out that different bacteria, fungi and algae are capable of producing physiologically active amounts of IAA.

In this study, the one factorial approach has been employed for optimization by varying one factor and keeping all the others fixed during the trial. The aim of this study was to increase IAA production and P-solubilization by varying different physiological parameters such as pH, temperature, carbon and nitrogen sources, so that we get the intended conditions at which the IAA production and P-solubilization is maximized. The present study reports optimization of growth parameters for IAA production and P-solubilization by plant growth promoting *Bacillus subtilis* KA(1)5r isolated from rhizosphere of *Aconitum heterophyllum*- an endangered medicinal herb of Western Himalayas. Information about *Bacillus subtilis* strain KA(1)5r with plant growth promoting attributes from *Aconitum heterophyllum* has not yet been reported. Hence, the results could be useful for better understanding of mechanism of plant growth promoting traits, which can help us to engineer this PGPR for agronomic interest.

Material and methods

The whole study was conducted at Microbiology laboratory and net house of "Department of Basic sciences," during 2013–2015. Study area was located at an altitude of 1,150 m above mean sea level. *Aconitum heterophyllum* a medicinal herb was used as the test plant. The present study was divided into following three major parts: isolation, screening and characterization of PGPR; pot experiment and optimization of growth parameters for P-solubilization and IAA production.

Isolation and characterization of rhizospheric soil (RS) and endorhizospheric (ER) bacteria from Aconitum heterophyllum

Sample collection and isolation of rhizobacteria

Composite rhizospheric soil and root samples were collected from *Aconitum heterophyllum* grown in its natural habitat from two locations at an altitude of 3300 to 5000 mnsl (Kangra and Lahaul) of Himachal Pradesh and were stored aseptically under refrigeration in plastic bags to ensure sufficient aeration and to prevent moisture loss until assaying of bacterial community structure.

Rhizospheric and root endophytic bacteria of *Aconitum heterophyllum* were isolated using serial dilution spread plate technique (Mehta *et al*., 2014) [7]. Total bacterial population were enumerated on nutrient agar and King's B agar medium after 24-48h of incubation and were expressed as colony forming units per gram of dry soil/root weight.

A total of 171 different morphotypes (96 RS and 75 ER) obtained on NA and King's agar medium were subjected to replica plating for one step screening of plant growth promoting traits; CAS medium for siderophore production (Schwyn and Neilands, 1987) [23], nitrogen free Jensen's medium for nitrogen fixation (Jensen, 1987) [24] and Pikovskaya's medium for phosphate solubilizing ability (Pikovskaya's, 1948) [25].

In vitro screening for plant growth promoting traits

Twenty most potent isolates showing P-solubilization, siderophore production and growth on nitrogen free medium were further characterized for additional traits associated with plant growth promotion.

Phosphate solubilization and nitrogen fixing ability

Population of P-solubilizers was screened on PVK agar medium incubated for 72h at 37 ºC. Formation of clear halozone around colony indicated P-solubilizing efficiency of bacterial isolates. For quantitative estimation, 50ml of sterilized PVK broth was inoculated with 1% of bacterial suspension and incubated for 72h at 37 ºC under shaken conditions. The culture supernatant was used for determination of the soluble phosphate (Bray and Kurtz, 1945) [26].

Nitrogen fixing ability was assessed in Nitrogen free medium using streak plate method; bacterial isolates showed growth on medium after 72h of incubation were considered as nitrogen fixers (Jensen, 1987) [24].

IAA Production

Quantitative production of IAA was determined using the colorimetric method described by Glick (Glick, 2012) [12]. Bacterial isolates were grown in Luria–Bertani broth (amended with 5 mM L-tryptophan, 0.065% sodium dodecyl sulfate and 1% glycerol) for 72 h at 37 ºC under shaken conditions followed by addition of 2 ml of Salkowski's reagent in 3ml of bacterial supernatant. The resulting solution was analyzed in a spectrophotometer at 530 nm and the IAA concentration was calculated using the equation obtained from the standard curve for commercial IAA.

Siderophore and HCN production

Bacterial isolates were assessed for siderophore production in CAS agar medium (Schwyn and Neilands, 1987) [23] for

qualitative estimation, formation of bright zone with a whitish (carboxylate), yellow (hydroxymate) or pink (catecholate) halozones around isolates in the dark blue medium signify siderophore production. For quantitative estimation absorbance was evaluated at 630nm.Hydrocyanic acid production was inferred by the qualitative method of (Baker and Schipper, 1987) [27].

Antifungal, Chitinolytic and Proteolytic activity

Antagonistic activity of selected PSBs was tested against *Fusarium oxysporum* and *Phytophthora capsici* using agar dilution confrontation experiment. Percent growth inhibition was calculated using formula proposed by Vincent, 1947[28].

The bacterial cultures were spotted on to the prepared minimal agar medium amended with 0.3% colloidal chitin and the plates were incubated at 30±2 °C for 4 days. Development of clear halo zone around the colony after addition of iodine was considered as positive for chitinase enzyme production (Robert and Selitrennik off, 1988) [29].

Clear halo zone produced around spot inoculated culture on skim milk agar plates was positive test of protease production examined after 48-72h of incubation at 28 ^{0}C (Fleming *et al*., 1975) [30].

In whole, isolate KA(1)5r showed maximum number of plant growth promoting traits, therefore was selected for molecular identification. On the basis of 16S ribosomal deoxyribonucleic acid (rDNA) sequencing, KA(1)5r was identified as *Bacillus subtilis*, accession number (MH202957).

Optimization of IAA production and P-solubilization parameters

Bacillus subtilis KA(1)r showed maximum amount of P-solubilization and IAA production under *in vitro* conditions, therefore was selected for optimization studies. The production of IAA and P-solubilization was optimized for selected isolate *Bacillus subtilis* KA(1)r by one factor at a time was employed in this study. Quantification of IAA production and P-solubilization was performed through the standard plot.

Effect of incubation time

Effect of incubation time on production of phytohormone and P-solubilization was studied by growing the bacterial strain at different incubation period (24, 48, 72, 96 and 120h). The optimum incubation time suited for the IAA production and P-solubilization was maintained for further experiments.

Effect of temperature

Temperature is also an important parameter for production of beneficial compounds since the growth of bacteria affected by low or high temperature. Thus, IAA production and P-solubilization was tested at 30, 35, 40 and 45 °C at 120 rpm.

Effect of pH

pH is one of the most important physicochemical parameters. A range of pH 5–9 was examined for its effect on IAA production and P-solubilization and the optimum pH was maintained for all further experiments.

Effect of inoculum size

Inoculum size of different population densities (0.5%, 1.0%, 1.5%, 2.0%, 2.5%) was added to LB and PVK broth for IAA production and P-solubilization, respectively and was determined after incubating flask at optimized incubation time, temperature and pH of the medium. The inoculum size showed maximum activity was used for all further experiments.

Effect of different concentrations

Different concentrations of tricalcium phosphate (0.1, 0.25, 0.5.75, and 1.0%) were used to determine the concentration at which the bacteria solubilize tricalcium phosphate effectively under liquid assay. For IAA production, different concentrations of tryptophan (0.1, 0.25, 0.5, .75, and 1.0%) were used to determine the concentration at which the bacteria produce IAA effectively.

The optimum concentrations were maintained for all further experiments.

Effect of medium composition

To elucidate the influence of each ingredient of the medium, IAA production and P- solubilization was estimated by deleting one component of LB and PVK medium, respectively.

Effect of *Bacillus subtilis* Ka(1)5r on plant growth promotion

Proficient isolate *Bacillus subtilis* Ka(1)5r was subjected to study their plant growth promoting potential on tomato seedlings under net house conditions for two months. 48h old culture of *Bacillus subtilis* Ka(1)5r grown in nutrient broth was used for bacterization of seeds (30 seeds). Treated seeds were then sown in each pot containing autoclaved mixture of sand, soil and farm manure (1:1:1) having one third moistened saturation capacity. After 3-4 days of seedling emergence thinning was done and five plants per pot were maintained. Eight replicate pots per treatment were placed in completely randomized design under net house conditions. Seeds dipped in autoclaved distilled water were kept as uninoculated control. Uninoculated control treatment was also kept in the same manner. The observations were recorded on% germination, vigor index, root-shoot length and root-shoot biomass.

Formula to calculate vigor index (Baki and Anderson 1973) [31]:

$$\text{vigor index} = (\text{mean root length} + \text{mean shoot length}) \times \text{germination (\%)}$$

Results and discussion

Isolation and characterization of rhizobacterial strain

Altogether**,** 171 rhizo and endo rhizobacterial isolates were isolated from composite rhizospheric soil and root samples of *Aconitum heterophyllum* grown in tribal regions of Kangra (Chotta Bhangal; 3500 m amsl, Bara Bhangal; 4000 m amsl) and Lahaul (Keylong; 3000 m amsl, Jispa; 3500 m amsl) valleys of Himachal Pradesh. Based on preliminary screening of P-solubilization, siderophore production and ability to fix nitrogen, twenty isolates were selected and were subjected to *in vitro* screening of multifarious plant growth promoting traits of direct and indirect mechanisms (Table 1). Among all the tested strains, isolate *Bacillus subtilis* KA(1)5r exhibited significant plant growth promoting attributes *viz.* P-solubilization, siderophore production, ability to fix nitrogen, IAA production and antagonistic traits of chitinase enzyme activity, amylase production, protease production, HCN production and antifungal activity against *Fusarium oxysporum* and *Phytophthora capsici*. Therefore, was selected for further studies. Different species of *Bacillus* as PGPR have been reported for several crops (Sharma *et al.,* 2017 [8]; Kumar *et al*., 2015 [9]; Mehta *et al*., 2013 [32]). *Bacillus* species

are capable of forming long-lived, stress tolerant spores and secreting metabolites that stimulate plant growth and prevent pathogen infection (Radhakrishan *et al*., 2017) [33].

Table 1: Screening of bacterial isolates for multifarious plant growth promoting traits

Isolates	P –solubilized (µg/ml)	Quantitative estimation (Percent Siderophore unit)***	Growth on nitrogen free agar medium	Indole-3-acetic acid (µg/ml)	HCN production*	Chitinase (E.I.)**	Protease (E.I)**	Antifungal activity	
								Fusarium oxysporum (%GI)***	*Phytophthora capsici* (%GI)***
La(2)5s	95.00	71.43	++	ND	++	-		+++	+
La(2)6s	130.00	69.42	+	32.00	-	+++	++	+++	+
Ka(3)4s	100.00	102.34	+++	ND	-	++	-	+++	+
La(3)4s	120.00	107.14	+++	ND	-	++	-	+	+
La(3)5s	160.00	29.98	++	ND	-	++	-	+	-
La(3)6s	100.00	45.67	++	ND	+++	-	++	-	++
La(3)8s	125.00	112.86	++	13.00	-	+	+++	++	+
Ka(4)1s	105.00	96.00	++	15.00	-	-	++	-	-
La(4)4s	100.00	23.45	++	24.00	++	-	-	++	-
La(5)1s	110.00	14.08	+++	ND	-	+++	++	-	++
Ka(5)3s	110.00	56.75	+++	4.00	-	-	+	-	-
La(6)5s	85.00	43.21	+++	21.00	-	-	-	+++	+
Ka(7)1s	95.00	78.91	++	11.00	+		+	+++	+
Ka(1)5r	170.00	142.14	++	34.00	+++	+++	+++	+++	++
Ka(3)1r	167.00	142.86	++	13.00	-	-	++	+	+
La(4)2r	135.00	66.51	+	20.00	-	++	-	+	-
Ka(4)3r	25.00	45.52	+	12.00	-	+	-	-	++
Ka(4)4r	55.00	102.86	+	22.00	+	+++	-	++	+
Ka(5)1r	26.00	71.43	++	14.00	++	+	++	-	-
Ka(6)6r	58.00	35.71	++	16.00	-	+	+++	++	-
$LSD_{0.05}$	6.23	2.23	-	1.85	-	-	-	-	++

ND: Not detected; $LSD_{0.05}$, least significant difference at P≤0.05
*(++) indicates orange brown colour, (+++) indicates dark brown colour, no activity (-)
**Values of enzyme index ranging from 1-1.5 (+); 1.5-3 (++); 3-3.5 (+++), no activity (-)
*** Values ranging from ≤60.00% (+); 60–70% (++); ≥70%, no activity (-)

Optimization of growth conditions for IAA production and P-solubilization

Effect of incubation period

Isolated bacteria *B. subtilis* KA(1)5r showed maximum production of IAA (34 µg/ml) and P-solubilization (170 µg/ml) under *in vitro* conditions, therefore was used for optimization studies. Incubation time was monitored upto 120 h of inoculation in LB medium and PVK medium for IAA production and P-solubilization, respectively.

Maximum P-solubilization (189 µg/ml) was obtained at 96 h of incubation with decline in pH from initial 7.0 to 4.68, corresponding to maximum viable count (87 × 10^6 cfu/ml) (Fig. 1(a). It has been reported in earlier studies that P-solubilization is mainly due to the production of microbial metabolites including organic acids which decrease the pH of the culture media and microbial metabolites are produced during late log phase (Mehta *et al.* 2014) [7]. Similar findings of highest P-solubilization (200.065 µg/ml) with the increasing hour of incubation upto 96h by *Pseudomonas* sp. has been reported earlier (George *et al*. 2012) [34].

IAA production by Ka(1)5r was also increased upto 96 h of incubation and highest amount of IAA (36µg/ml) was produced after 96 h of incubation with corresponding viable count of 59×10^6 cfu/ml as well as pH 8.12 from initial pH (Fig. 1(a). The decrease in IAA production during late log phase might be due to the release of indole acetic acid releasing enzymes such as indole acetic acid oxidase and peroxidase that has been reported earlier by Datta and Basu, 2000) [35].

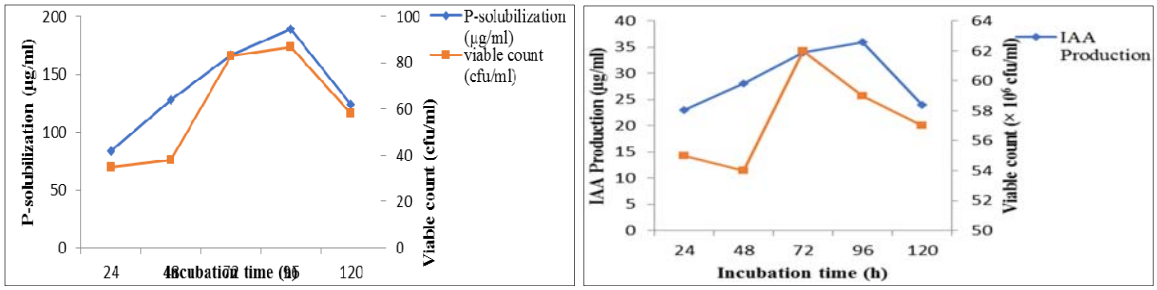

Fig 1a: Optimization parameters for P-solubilization and IAA production: (a) Effect of incubation time.

Effect of temperature

The effect of temperature was studied in the range of 30-45 °C whereby maximum yield of P-solubilization (184 µg/ ml) was observed at 37 °C corresponding to maximum viable count (89 × 10^6 cfu/ml) (Fig. 1(b). Present results are in line with Malleshwari and Bhagyanarayana, 2013 [36], where optimum temperature for P-solubilization was at 35 °C. Maximum IAA production (37 µg/ml) was recorded at 35 °C

with subsequent increase in viable count (61×10^6 cfu/ml) and corresponding pH of 8.15 (Fig. 1(b). Dasgupta *et al*., 2015 [37] had studied the effect of temperature on IAA production and his studies corresponds to our results where the maximum increase in IAA was upto 37 °C of incubation temperature.

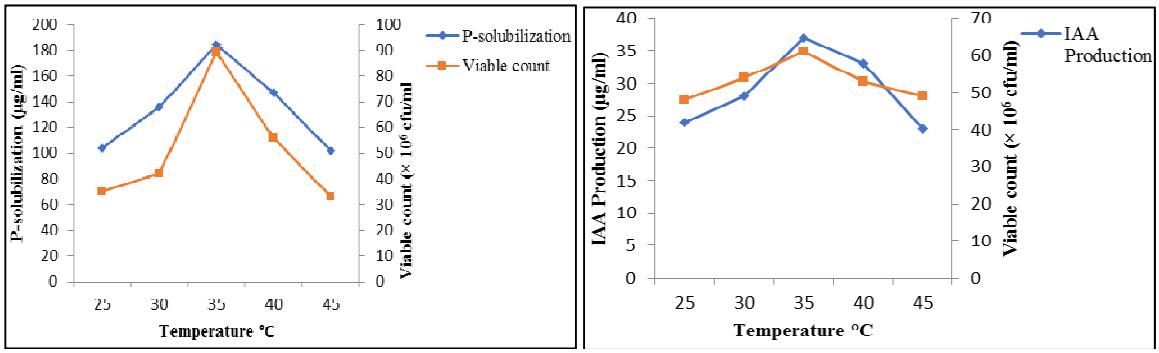

Fig 1b: Optimization parameters for P-solubilization and IAA production (b) Effect of temperature

Effect of pH

A number of physiological and metabolic processes taking place in the rhizosphere can be affected by soil pH and metal cations present in the vicinity, therefore, impact of pH range of 5–9 was also examined for P-solubilization and IAA production. P-solubilization Fwas monitored upto pH range of 5-9 and maximum was recorded for pH 6.0 (197 µg/ ml) and then solubilization decreased with subsequent increase in pH (Fig. 1(c). There are several reports of highest P-solubilization at neutral pH (Mehta *et al.* 2013 [32]; Nautiyal *et al*. 2000 [38]; Mehta and Nautiyal, 2001 [39])

IAA production was increased from pH 5-8 and was highest at pH 8 (37 µg/ ml) with maximum viable count (61× 10^6 cfu/ml) (Fig. 1 (c). It was also observed by Dasgupta *et al.*, 2015 [37] that the acidic or high alkaline conditions are not suitable for IAA production, in same context isolate Ka(1)5r showed maximum IAA production at neutral pH (8.0) indicating that neither acidic nor alkaline conditions promotes IAA production.

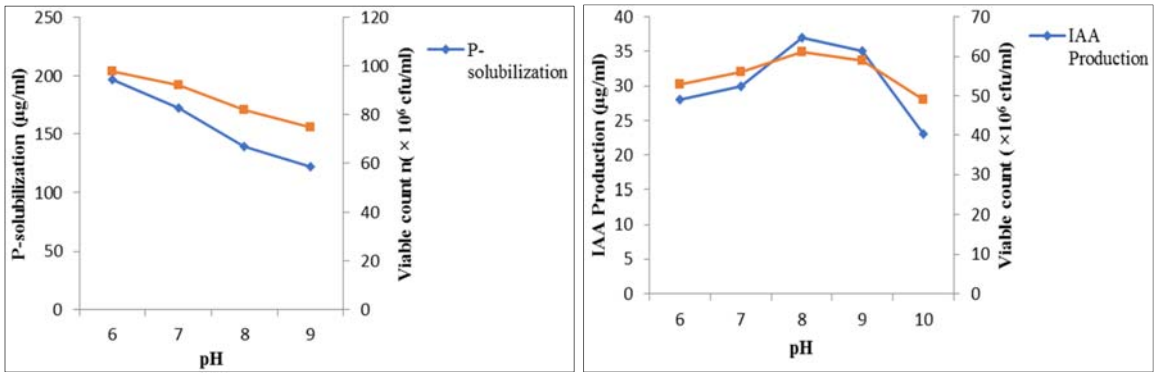

Fig 1c: Optimization parameters for P-solubilization and IAA production(c) Effect of pH

Effect of inoculum size

Impact of inoculum size was studied by inoculating different concentrations (0.5%, 1%, 1.5%, 2.0%, 2.5%) of inoculums in LB and PVK broth (Fig. 1(d). For P-solubilization maximum value (181 µg/ ml) was obtained in the PVK broth inoculated with 1 per cent culture while in case of IAA, the production was increased with increase in inoculums size and maximum (39 µg/ ml) was obtained in LB broth inoculated with 2 per cent culture.

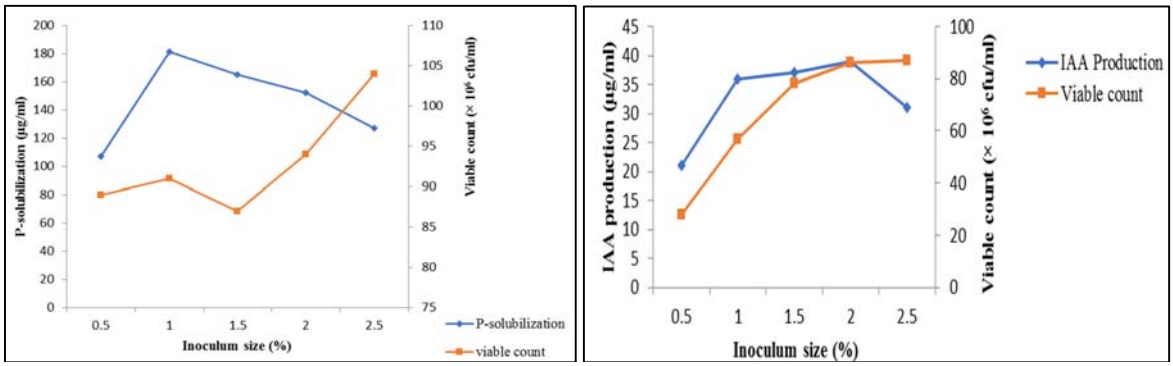

Fig 1d: Optimization parameters for P-solubilization and IAA production (d) Effect of inoculums size

Effect of concentration

A gradual increase in IAA production and P-solubilization by *B. subtilis* KA(1)5r was also observed with the increase in tryptophan (0.1, 0.25, 0.5, 0.75, 1.0%) and TCP concentration (0.1, 0.25, 0.5, 0.75, 1.0%) (Fig. 1(e). At concentration of 1 per cent tryptophan and TCP, maximum IAA production (42 µg/ ml) and P-solubilization (192 µg/ ml) was recorded, respectively.

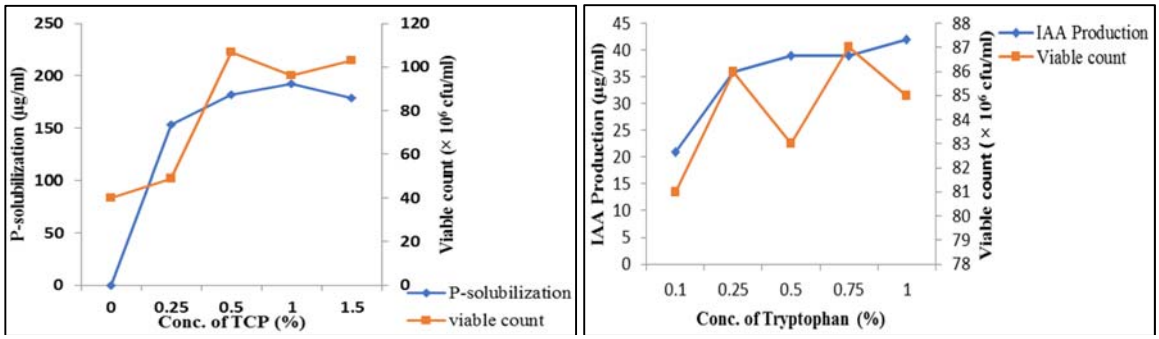

Fig 1e: Optimization parameters for P-solubilization and IAA production (e) Effect of concentration of tricalcium phosphate and tryptophan

Effect of different ingredients

To elucidate the influence of each ingredient of the medium on P-solubilization and IAA production, one component at a time was deleted in PVK and LB medium (Fig. 1(f). In case of P-solubilization, maximum value (197 µg/ml) was observed in the absence of yeast extract corresponding to the maximum viable count (104 × 10^6 cfu/ml) with decrease in pH from 6.00 to 4.65, while no P-solubilization was recorded in the medium lacking glucose and tricalcium phosphate. Similar results were observed by Mehta *et al*., 2013 [32] suggesting that the P-solubilization was dependent on presence of glucose in the medium and exclusion of yeast extract had no significant effect on TCP solubilization.

Yeast extract plays an important role in IAA production; the current statement supports our results in that no IAA production was found in LB medium lacking yeast extract. Whereas, NaCl had no significant effect on IAA production and showed maximum IAA production (35 µg/ml) with corresponding maximum viable count (73× 10^6 cfu/ml) and pH 8.11. Deshwal *et al*., 2013 [40] showed optimum IAA production at NaCl concentration of 0 to 0.75 per cent in the medium and observed no significant effect on IAA production in absence or presence of NaCl, which supports our results.

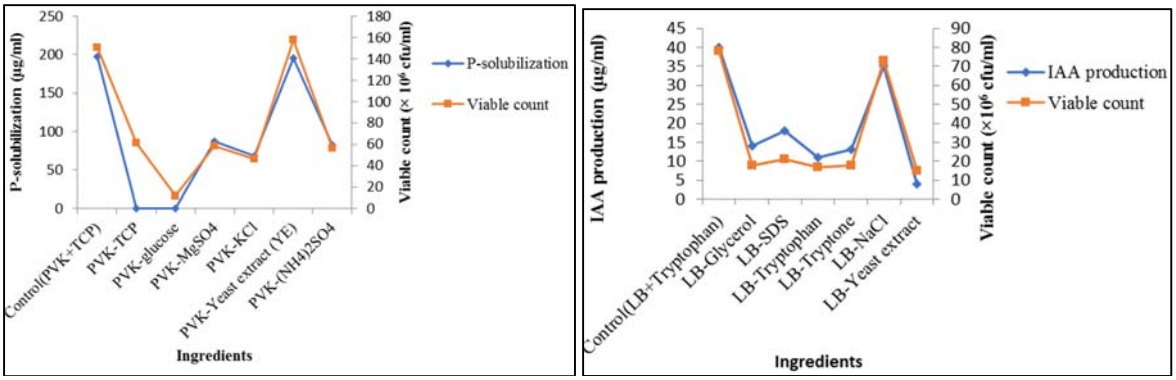

Fig 1f: Optimization parameters for P-solubilization and IAA production (f) Effect of various ingredients

In vitro plant growth promotion studies

A large body of evidence suggests that PGPR enhances the growth, seed emergence as well as crop yield, and contributes to the protection of plants against certain pathogens and pests Dey *et al*., 2004 [41]. Therefore, in the present study, inoculation effect of *Bacillus subtilis* KA(1)5r was evaluated on tomato seedlings. Plant growth promotion studies under net house conditions revealed that tomato seeds treated with isolate *Bacillus subtilis* KA(1)5r resulted in maximum seed germination, higher root length, shoot length, root biomass, shoot biomass and vigor index over uninoculated control (Table 2). Statistically significant per cent increase in germination (7.41%), root length (14%), shoot length (41%), root biomass (75%), shoot biomass (82%) and vigor index (48%) was observed in seeds treated with *Bacillus subtilis* KA(1)5r over uninoculated control, this might be attributed to inoculation effect on increasing soil nutrients available to the plant, production of phytohormones, increasing plant metabolism, enhancing number and action of beneficial rhizobacterial population or by suppressing phytopathogens (Perez-Montano, 2014) [42].

The findings of the present investigation highlighted that the isolated strain has great potential to enhance soil fertility and plant growth promotion through the production of IAA and P-solubilization. However, this assessment of plant growth promotion, further field studies are required for recommending the strain as bio- inoculants for the crops that are exposed to several biotic and abiotic stresses.

Table 2: Effect of seed treatment with liquid formulation of isolate *Bacillus subtilis* KA (1)5r on growth parameters of tomato seedlings under net house conditions (60 days after sowing)

Isolates	Germination (%)*	Root length (cm)	Shoot length (cm)	Root dry weight (mg/plant)	Shoot dry weight (mg/plant)	Vigor Index
Uninoculated control	90.00 (9.48)	6.60	54.00	40.00	270.00	5454.00
Bacillus subtilis KA(1)5r	96.67 (9.83)	7.80	76.00	70.00	490.00	8100.94
$CD_{0.05}$	4.90	1.25	3.82	8.21	14.61	34.34

*Figure in Parenthesis () are square root transformed values

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