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Evaluation of *Bacillus thuringiensis* Snkr10 as a Potent Plant Growth Promoting Phyllospheric Bacterium Isolated from Spinach (*Spinacia oleracea*) Plants

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Authors' contributions

This work was carried out in collaboration between both authors. Authors NS and BSS designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Author NS managed the analyses of the study and the literature searches. Both authors read and approved the final manuscript.

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Original Research Article

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ABSTRACT

The phyllospheric microorganism used leaf surface of plants as habitat and help to maintain the plants growth by behaving as plant growth promoting bacteria (PGPB). SNKr10 is a gram positive and rod shaped spinach phyllospheric bacterium with 100% identity to *B. thuringiensis* (Accession no: KU569966). This bacterium showed various plant growth promoting (PGP) attributes like phosphate solubilization, Indole acetic acid (IAA), siderophore, NH_3, HCN production and *in vitro* biological nitrogen fixation (BNF). Result also showed that this bacterial strain exhibit positive effect on the growth parameters of *T. foenum-graecum* (methi) under *in vitro* seed germination experiment and pot trial of *V. radiate* (mung bean). These results suggested that *B. thuringensis* has an excellent potential to be used as bio-inoculant for agriculture.

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1. INTRODUCTION

Phyllosphere is the leaf surfaces of a plant that is used as a habitat for microorganisms. Plant growth promoting bacteria (PGPB) not only inhabit on plant surface, also on the root region (majority). PGPB are beneficial bacteria, having colonization ability and ability to enhance plant growth and protect them from disease and abiotic stress. The beneficial effects of these bacteria on plant growth can be direct or indirect. PGPB may promote plant growth directly usually by either facilitating resource acquisition or modulating plant hormone levels e.g. IAA, cytokinins and gibberellins; biological nitrogen fixation and the assemblage of unavailable nutrients such as insoluble form of phosphorus and other mineral nutrients. Indirect mechanism is performed by decreasing the inhibitory effect of various pathogens on plant growth through secretion of hydrolytic enzyme (chitinase, protease), siderophore, HCN production, antibiotic like phenazine, phenazine, kanosamine, oligomycin A etc. [1,2]. Various *Bacillus* ap. produced antimicrobial compound which showed toxic effect against pathogenic microbes like Iturin D, Iturin A, Surfactin, TasA-(protein with broad spectrum antibacterial activity) and mycotoxins are produced by *B. subtilis* [3,4].

Most of the PGPB enhance the growth of plants by several mechanisms. The mechanisms can probably be active simultaneously or sequentially of different stage of plant growth. Some examples of the mechanisms are (a) Increased mineral nutrient solubility and nitrogen fixation, making nutrient available to the plants, (b) phythohormone production (c) antagonism against phytopathogenic pathogens by producing siderophores, chitinase, HCN e.g. *B. subtilis, B. cepacia etc.*, help to prevent the plant from pathogenic microbes by HCN production [5,6].

The spinach (*S. oleracea*) is an important medicinal plant that has various nutritional and health benefit values. Various bacillus sp. has reported earlier as PGPB like *Bacillus amyloliquefaciens* NBRISN13 (SN13) helps in rice plant growth during stress conditions [7]. Some other Bacillus sp. including *Bacillus pumilus* ES4, *B. licheniformis, B. polymyxa, B. subtilis, B. putida, B. cereus* etc. showed their PGPB role in the growth of chickpea, pigeon pea, wheat, tomato, soybean crops [8,3].

B. thuringiensis SNKr10 is one of bacillus sp. which is gram positive rod shaped, endospore forming non-pathogenic strain of bacteria isolated from spinach phyllosphere. Our main objective of this research is to study the beneficial PGP properties of *B. thuringiensis* SNKr10. So that it can be used as potent bio-fertilizer for the sustainable agriculture. This bacterium plays important role in agriculture like phosphate solubilization, IAA production, HCN, ammonia and *in vitro* BNF. The role of this bacterial strain on the growth of plants using *in vitro* seed germination assay as well as pot house experiment was also studied.

2. MATERIALS AND METHODS

2.1 Sample Collection and Isolation

Spinach leaves were collected from different fields of Haryana and Punjab including Chika, Shahabad, Karnal, Kurukshetra, Kapiyal and Sangroor fields, in India. Fresh leaves were stored in sterile propylene bags, washed with sterile distilled H_2O and then vortexed for 10-20 minutes [9]. Serially dilutions (10^{-1}-10^{-5}) were also prepared and about 100 µL of each dilution was spreaded on nutrient agar media for 24-48 h of incubation at 35-37℃. The isolated bacterial cultures were preserved in nutrient slants for the study of PGP properties [10]. SNKr10 bacterial study is discussed here in detail.

2.2 Characterization of Bacterial Isolates

The bacterial isolate was examined for their morphological and biochemical characteristics using Bergey's manual of systematic bacteriology [11]. Gram staining and endospore staining was also performed for identification of bacterial isolate [12].

DNA was isolated from each sample using Biopure™ kits (Bioaxis DNA Research Centre) for Bacteria genomic DNA isolation. Bacterial isolates were analyzed by PCR using 16S universal primer set:
F= AGAGTTTGATCHYGGYTYAG;
R = ACGGCTACCTTGTTACGACTT [13].

PCR Conditions; 1 cycle: 94℃ temperature for 5 minutes (Initial denaturing); 35 cycles: 94℃ temperature for 60 seconds (denaturing); 53℃ temperature for 45 seconds (annealing); 68℃ temperature for 90 seconds (extension); Cycle:

68℃ temperature for 10 minutes (final extension)

PCR product was amplified and electrophoresis was performed using 1% agarose gel in TAE buffer and then visualized with EtBr stain. PCR product was then washed with sodium acetate and 70% of ethanol for purification and then eluted from the gel. Forward and reverse sequencing reactions of PCR amplicon were carried out by using ABI 3730XL sequencer to obtain the sequence. The assembled DNA sequence was then used to obtain BLAST with the nucleotide data base of NCBI.

2.3 Screening of Bacterial Isolate for PGP Attributes

2.3.1 Phosphate solubilization

The phosphate solubilization of bacterial isolates was performed on pikovskaya's agar medium having glucose 10 gL^{-1}: $Ca_3(PO_4)_2$ 5 gL^{-1}; $MgCl_2 \cdot 6H_2O$ 5 gL^{-1}; $(NH_4)_2SO_4$ 0.1 gL^{-1}; $MgSO_4 \cdot 7H_2O$ 0.25 gL^{-1}; KCl 0.2 gL^{-1}; bromothymol blue dye and pH-7.0 [14]. The bacterial cultures were streaked and incubated at 35-37℃ for 3-5 days and observed for diameter of zone of phosphate solubilization. The solubilization efficiency and phosphate solubilization index were also calculated by the following formula:

$$\text{Solubilization efficiency (Pi)} = \frac{\text{Solubilization diameter}}{\text{Colony diameter}} \times 100$$

$$\text{Phosphate solubilization index (SI*)} = \frac{\text{Colony diameter+Clearing zone}}{\text{Colony diameter}}$$

PS was quantitatively estimated by inoculating respective bacterial isolate in Pikovskaya's broth containing tri-calcium phosphate (0.5%) and incubated at 35-37℃ for 10 days using stannous chloride method [14]. The inoculated broth was centrifuged and about 10 mL clear supernatant was mixed with equal volume of ammonium molybdate solution by constant shaking to avoid CO_2. Distilled water at the concentration of 2 mL was also added in it along with addition of 0.25 mL freshly prepared $SnCl_2$ solution and final volume (50 mL) was prepared using distilled water. The colour intensity and change in pH was analysed at 630 nm. Effect of different concentration of phosphate (0.2-2%) was also estimated after incubation for 5 days by using the same method [14].

2.3.2 Ammonia production

The respective fresh bacterial culture was inoculated in 10 mL peptone water and incubated at 30-37ºC for 48-72 h. About 0.5 mL of Nessler's reagent was then added and allowed to settle at room temperature. The appearance of brown colour indicated the positive reaction for ammonia production [12].

The ammonia production was quantitatively analysed by inoculating the fresh bacterial culture into peptone water at 35 ±2℃ for 1-7 days. The culture medium was centrifuged for 15 minutes at 10,000 rpm. The supernatant was collected at the interval of 24 h. About 1 mL of Nessler's reagent was added to I mL of supernatant and then 10 mL of total volume was made by using distilled water. The yellow colour was detected at 630 nm by using spectrophotometer. The concentration of ammonia so produced was calculated using standard curve which was prepared by taking ammonium sulphate in the range of 0.1-1.0 mMol mL^{-1} [15].

2.3.3 Indole-3-acetic acid (IAA) production

The bacterial isolate was inoculated in Luria broth containing L-tryptophan (0.1%) and incubated at 35-37℃ for 48-72 h. The culture broth was then centrifuged at 10,000 rpm for 10 minutes and two drops of o-phosphoric acid were added into 2 mL of supernatant along with 4 mL Salkowski reagent (50 mL, 35% of perchloric acid, 1 mL of 0.5 m $FeCl_3$ solution). The development of pink colour within 2 h at room temperature indicated the positive IAA production ability of bacterial strain [16,17].

For the quantitative estimation of IAA, the bacterial isolate was inoculated in LB broth containing different concentration of L-tryptophan (20-500 µg mL^{-1}). It was incubated for 3 days at 35-37℃. The cultural conditions like concentration of L-tryptophan and incubation period were optimized following the strategy of keeping one parameter variable at a time. The incubation period was tested up to 7 days. After centrifugation about 1 mL supernatant was mixed with 4 mL of salkowski reagent and IAA production was detected within 30 minutes using spectrophotometric study at

530 nm. The concentration of IAA production was estimated using standard curve of IAA [16,17].

2.3.4 HCN production

The production of HCN was performed using nutrient agar medium amended with glycine (0.44%). Sodium carbonate (2%) was prepared in 0.5% picric acid solution. Whatman's filter paper No. 1 was soaked in this Sodium Carbonate solution and placed along with the top lid of petri plate. After inoculation the bacterial inoculated plates were sealed with parafilm and incubated at 35-37ºC for 3-5 days. Development of orange/red colour indicated the positive test [18].

2.3.5 *In vitro* biological nitrogen fixation

In vitro BNF was observed by streaking the bacterial culture on nitrogen free Jensen's media using bromothymol blue stain (BTB) as an indicator dye and incubated at 35-37ºC for 24-48 h. The yellow coloured zone around the colonies indicated the positive *in vitro* BNF [19].

Nitorgen fixing activity was quantitatively estimated by inoculating the bacterial culture in nitrogen free broth medium emended with sucrose (3% w/v) and incubated at 35-37ºC for 1-7 days. The 2-10% of sucrose concentration was also used in order to check the effect of its concentration on *in vitro* BNF and O.D. was observed at 630 nm with minor modification in method of [19]. The % *in vitro* BNF was calculated as:

(%) *in vitro* BNF =

$$\frac{\text{O.D. of Reference} - \text{O.D. of cell free filtrate}}{\text{O.D. of References}} \times 100$$

2.3.6 *In vitro* seed germination assay

Seeds of *T. foenum-graecum* (methi plant) were used for assay and surface sterilized using 0.1% of sodium hypochlorite solution for 2-3 min. these seeds were then washed for three times with sterile distilled water to remove the sodium hypochlorite solution [20]. The seeds were bacterized with the respective bacterial isolate for 1-2 h and kept on 1% soft agar in petri plates and incubated for 3-5 days at 25-28℃ in the dark for seed germination. The untreated seeds were used as control. Various growth parameters like seedling height, % seed germination, root/shoot length, wet/dry weight and vigor index were calculated as:

Seed germination (%) =

$$\frac{\text{No. of germinated seeds}}{\text{Total no. of seeds}} \times 100$$

Vigor index = % Seed Germination × Seedling length (cm)

2.4 Pot House Experiment

Pot house experiment was performed by treating the seeds with selected bacterial isolates having superior characteristics. The seeds of *V. radiata* were sown into pots filled with sandy loamy sterile soil. About 30 surface sterilized seeds were bacterized with 24 h old bacterial culture (10^8 CFU mL^{-1}). for 1 h and used for sowing in pots at 30-40℃ under aerobic conditions. The various parameters were recorded/calculated upto 21-30 days of *V. radiata.* During the experiment, the pots containing treated and un-treated seeds were irrigated with sterilized water every day [21].

3. RESULTS AND DISCUSSION

3.1 Isolation and Identification of Bacterial Isolate

We isolated 200 bacterial isolates using serial dilution technique on nutrient agar media. These bacterial isolates were identified on the basis of size, colour, elevation, shape and pigmentation *etc.* The pre-screening involved the overall study of all the given PGP attributes that are discussed below (IAA, ammonia, phosphate solubilization, BNF etc.). We observed that 15 bacterial isolates from these 200 bacterial isolates showed proficient plant growth promoting activities [22]. We discussed here about the detailed PGP properties of SNKr10.

SNKr10 is irregular in shape, dispersed elevation, with flat surface and creamish coloured bacterial colonies. Using gram and endospore staining it was observed that SNKr10 is a gram positive rod shaped and endospore forming bacilli (Fig.1.1; Fig. 1.2; Fig. 1.3). About 89 bacterial isolates were isolated from wheat phyllosphere for the study of bacterial diversity as well as their PGP attributes [23] that favour our findings of isolation and PGP study of spinach phyllospheric bacteria.

The SNKr10 bacterial isolate was found to be positive for Voges Prausker (VP),

nitrate reduction test, starch, citrate utilization. Isolate SNKr10 also produced positive carbohydrate fermentation reaction for maltose and glucose and negative test for H_2S production *etc.*

The molecular characterization of SNkr10 was analysed on the basis of 16S rRNA sequencing (Table 1). Blast information as well as phylogenetic tree was formed (having scale of 0.003) (Fig. 2; Fig. 3).

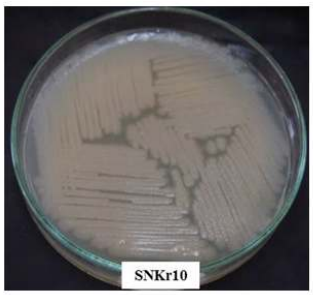


Fig. 1.1

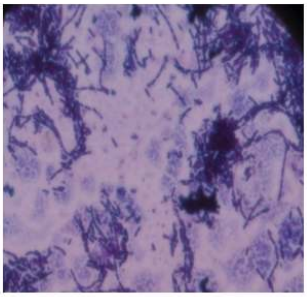


Fig. 1.2

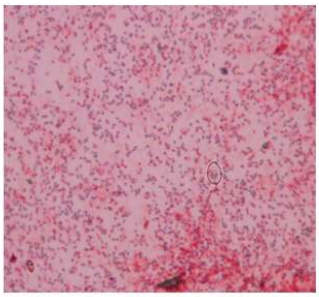


Fig. 1.3

Fig. 1.1. Streaking of SNKr10 on nutrient agar plate
Fig. 1.2. Gram staining of SNKr10
Fig. 1.3. Endospore staining of SNKr10

Table 1. Molecular characterization of selected bacterial isolates

Strain	16 rRNA	Class of bacteria	Closest relative	Similarity	Accession number
SNKr10	1510 bp	*Bacillus thuringiensis* SNKr10	*Bacillus thuringiensis*	100%	KU569966

SNKr10 Gene sequence detail

gi|979484805|gb|KU569966.1| *Bacillus thuringiensis* strain SNKr10 16S ribosomal RNA gene, partial sequence

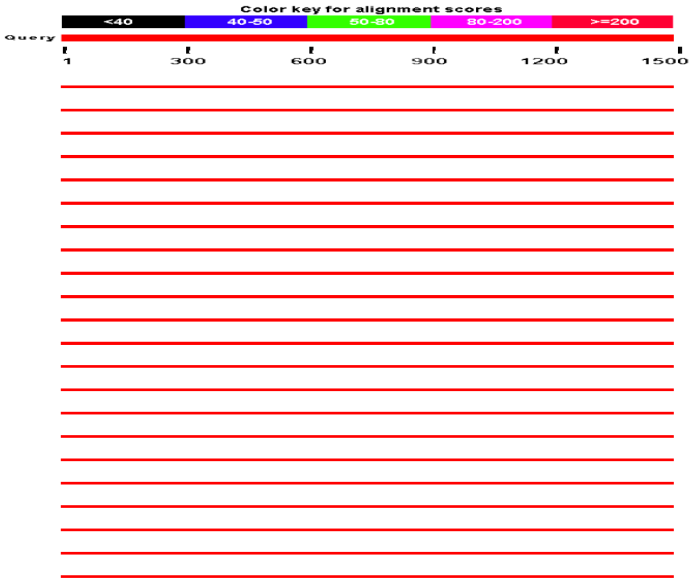


Fig. 2. Alignment of consensus sequence using BLAST for *Bacillus thuringiensis*

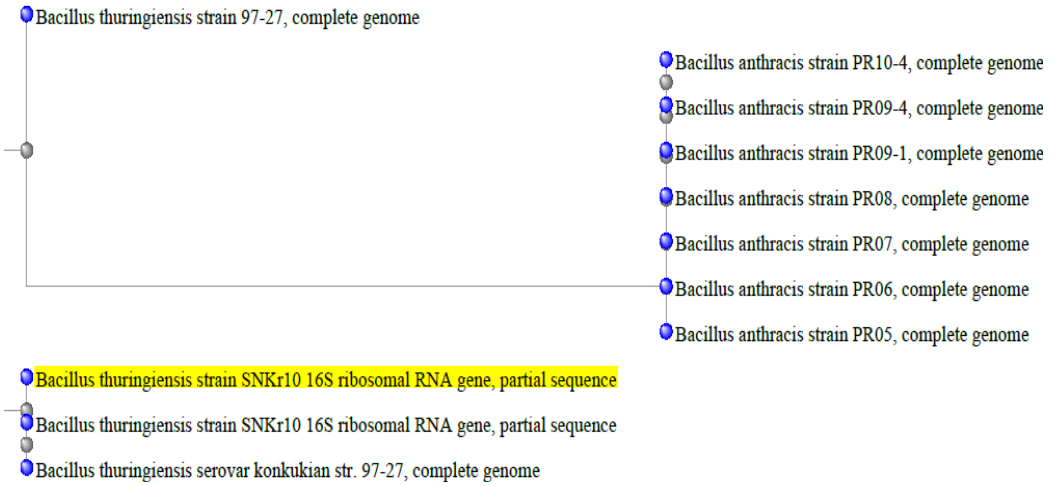


Fig. 3. Phylogenetic tree of *Bacillus thuringiensis* SNKr10

3.2 Screening for PGP Attributes

Various PGP attributes like Phosphate solubilization, IAA production, ammonia production; *In vitro* BNF, HCN production etc. were studied as.

3.2.1 Phosphate solubilization

It was observed that SNKr10 bacterial isolate was exhibiting phosphate solubilization by forming a clear halo zone around the colonies on Pikovskaya (PVK) agar plate (Fig. 4.1). The diameter of halo zone and phosphate solubilization efficiency (PSE) observed was about 1.8 cm and 144.58% respectively. Quantitatively, along with different time incubation (5 g L^{-1} of tricalcium phosphate), we observed that SNKr10 solubilized 150 µg mL^{-1} of phosphate and drop in pH (3.88) of PVK broth was also observed (Fig. 4.2; Fig. 4.3). It was observed that the solubilized phosphate leads to the reduction in the pH of the medium due to production of some acidic compound during phosphate solubilization [24]. Phosphate solubilization ability in lower/higher concentration was also reported from bacteria that was isolated from mangrove plants like PSB-26 bacterial isolate, solubilized maximum of 48.70 µg mL^{-1} phosphate after 6 days of incubation [24]. Goswami et al. [25] reported that 13 mg mL^{-1} of Ca_3PO_4 was solubilized by *Pseudomonas aeruginosa* BS8 which is also significant solubilization efficiency as compared to SNKr10. This variation in solubilization may be due to different environmental conditions.

We also observed the phosphate solubilization with varying concentration of TCP (2-20 g L^{-1}) in the PVK broth medium and drop in pH along with phosphate solubilization. The maximum of 120 µg mL^{-1} phosphate was solubilized by SNKr10 by utilizing 8 gL^{-1} of TCP and significant drop in pH was 3.39 (Table 2).

Similarly in the present investigation, a significant decline in the pH of the culture medium by all the strains was observed during mineral phosphate solubilization, which may be due to the production of organic acids as reported earlier by several researchers [26,27]. This decrease in pH level during phosphate solubilization was supported by findings of Yadav et al. [28]. They reported that phosphate solubilization by *A. niger* (PGPF) in PVK broth leads to the decrease in pH to 3.08.

3.3 Ammonia and IAA Production

Ammonia production play important role in growth of plants by converting atmospheric nitrogen into ammonia during nitrogen cycle. The useful PGPB also breaks down nitrogenous materials (peptones) into ammonia which were then released into soil and taken up by plants as a nutrient improvement of plant growth and development. This NH_3 helps in synthesis of proteins, enzymes, chlorophyll, RNA and DNA, and therefore improve the growth of plants [29]. The SNKr10 produced significantly 145.6 µg mL^{-1} of ammonia after 4 days of incubation (Fig. 5; Table 3). *P. aeruginosa* strain BS8 also produced maximum of 27 mg mL^{-1} of ammonia [25]. Along with this, Dutta et al. [30] reported

that *Rhizobacteria* named *Pseudomonas aeruginosa* strain KH45 isolated from tea plants are able to produce 4.9 µmol mL $^{-1}$ of ammonia after 72 h of incubation and significantly increased the biomass plants.

IAA is an important plant growth promoting hormone that helps in growth and development of plants by cell division, development of root, differentiation of phloem and xylem tissues, shoot growth, regulation of photo- and gravi-tropisms, apical dominance, delayed leaf senescence stimulation of growth in certain flower parts, stimulate seed germination; initiates root formation; biosynthesis of various metabolic compounds and it also helps in resistance towards stressful conditions [31,32,33].

SNKr10 produced maximum of 65.0 µg mL^{-1} of IAA after 5 days of incubation and at 80 µg mL^{-1} of L-tryptophan, SNKr10 produced 56.8 µg mL^{-1} of IAA (Fig. 6; Table 4; Fig. 7). The maize endophyte bacterium *Pantoea* sp. (FF34) also produced maximum of 95.34 µg mL^{-1} of IAA, when amended with 0.1% L-tryptophan and 10.33 µg mL^{-1} IAA in absence of L-tryptophan containing broth medium [34]. SNKr10 bacterial capacity for IAA production varies from these findings may be due to different microbial habitat and environmental conditions.

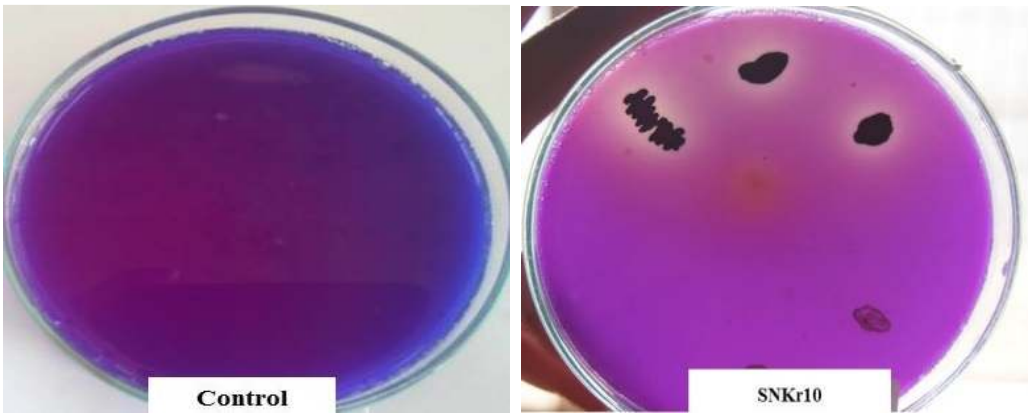


Fig. 4.1. Phosphate solubilization by SNKr10

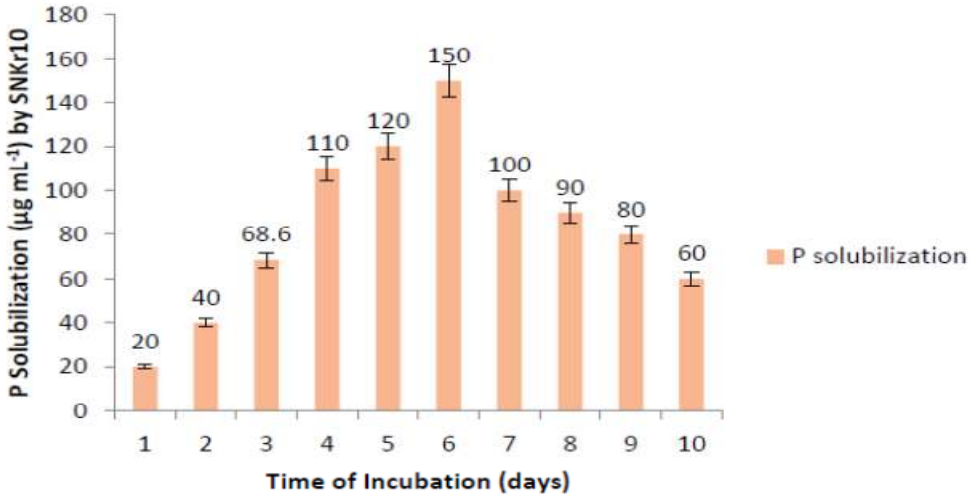


Fig. 4.2. Phosphate solubilization (µg mL^{-1}) by SNKr10 bacterial isolate along with time of incubation (days)

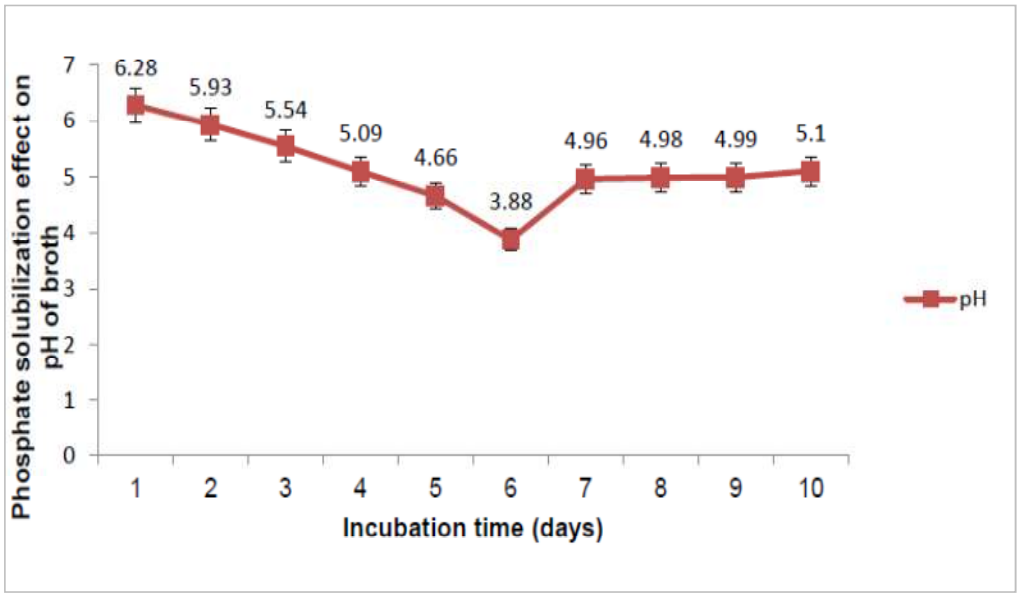


Fig. 4.3. Phosphate solubilization effect on pH of broth along with time incubation (days)

Table 2. Effect of SNKr10 bacterial isolate on phosphate solubilization and pH of broth along with time incubation

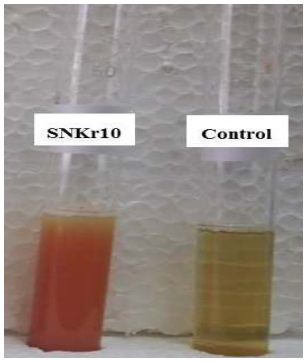
Tricalcium phosphate (g L^{-1})	Amount of phosphate solubilized by SNKr10 (µg mL^{-1})	pH effect during phosphate solubilization
	(Mean ± S.D)	(Mean ± S.D)
2	80±0.01	6.1±1.10
4	80±0.01	4.98±0.02
6	90±1.00	4.97±0.10
8	120±1.12	3.39±0.02
10	89±1.00	4.99±0.11
12	64±0.03	4.89±0.03
14	60±0.31	4.73±0.10
16	58.9±0.11	5.03±0.02
18	55.2±0.10	5.11±0.04
20	30±0.12	5.93±0.01

Singh et al. [35] also favoured our findings by reporting that PB10 bacterial strain of *Advenella* sp. produced maximum of 35.03 μg mL^{-1} of IAA followed by PB06 strain having 26.0 μg mL^{-1} of IAA after an incubation period of 24 h after that it started decreasing from 24-72 h.

3.4 HCN Production and *In vitro* BNF

HCN is a secondary metabolite produced by PGPB that act as biological control agents because it helps to degrade the metabolic activity of pathogenic microbes and indirectly influences plant growth. HCN also help to degrade the weeds, blocks the cytochrome oxidase pathway and is highly toxic to all pathogenic microorganisms [36,37].

HCN production by SNKr10 was confirmed by changing yellow coloured (saturated with picric acid solution) filter paper in to deep orange (Fig. 8). Agbodjato et al. [38] reported that various PGPR including five *Bacillus* sp. (*B. polymyxa, B. pantothenticus, B. anthracis, etc.*), three *Pseudomonas* sp. and *Serratia marcescens* that were isolated from maize rhizospheres in central and northern Benin showed the HCN production and exhibit the inhibition effect against plant pathogens and therefore favour our findings.



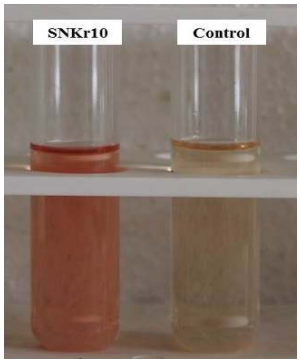


Fig. 5. Ammonia production **Fig. 6. IAA production**

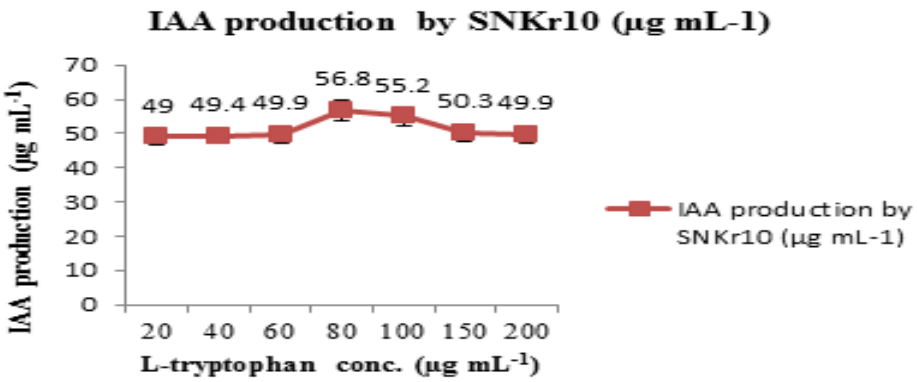


Fig. 7. IAA production by SNKr10 with L-tryptophan concentration

Table 3. Ammonia production by SNKr10 with time incubation

Incubation time (days)	Ammonia Production (µg mL^{-1}) (Mean ± S.D)
1	11.2±0.21
2	66.06±0.24
3	142.7±0.31
4	145.6±0.01
5	145±0.04
6	140.05±0.04
7	66.06±0.21

Table 4. IAA production by SNKr10 with time incubation

Incubation time (days)	IAA production (µg mL^{-1}) (Mean ± S.D)
1	30.2±0.04
2	46.06±0.24
3	52.7±0.21
4	55.6±0.01
5	65±0.21
6	46.05±0.14
7	40.06±0.12

In vitro BNF is also an important PGP attribute that promote growth by providing nitrogen as a useful form to the plants for their growth. BNF by SNKr10 was also confirmed by forming yellow halo around colony and about 20 mm of BNF yellow zone was formed by SNKr10 in nitrogen free media (Fig. 9). Quantitatively, SNKr10 produced significant % BNF with time incubation and sucrose concentration in nitrogen free jenson's broth medium (Fig. 10; Table 5). Verma et al. [39] reported in favour of our findings by introducing that several bacterial strains like *Achromobacter, Alcaligenes, Bacillus, Delftia, Providencia, Pseudomonas, Rhodobacter, Salmonella, A. faecalis, B. subtilis, D. acidovorans* and *M. mesophilicum* showed *in vitro* BNF and bacterial strain IARI-NIAW2-29 fixed maximum of 87.5% *in vitro* BNF in wheat plant and help to promote the growth of plant.

3.5 *In vitro* Seed Germination Assay

In present investigation, we observed that SNKr10 showed significant PGP activities that are discussed above (IAA, ammonia, HCN, phosphate solubilization, *in vitro* BNF) and

further used for study of their effect on the growth of *T. foenum-graecum* seedlings [Fig. 11]. These bacterial treatments to the seeds had shown a stimulatory effect on all seed germination parameters of *T. foenum-graecum* as compared to the untreated plants (Table 5). Earlier literature report of Zhao et al. [40] also supported our findings by reporting that *Burkholderia phytofirmans* strain PsJN helps to increase the biomass and growth of several plant species (*Solanum tuberosum* L.) by improving the root and shoot growth by inducing various phytohormone.

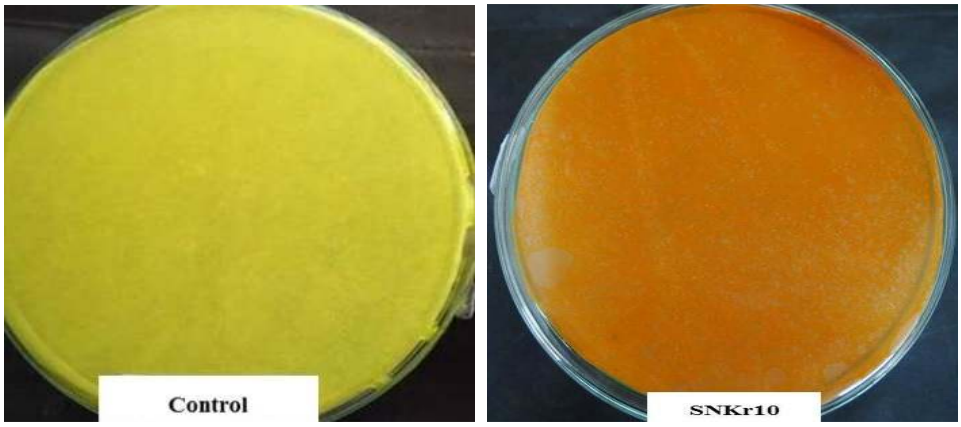


Fig. 8. HCN production

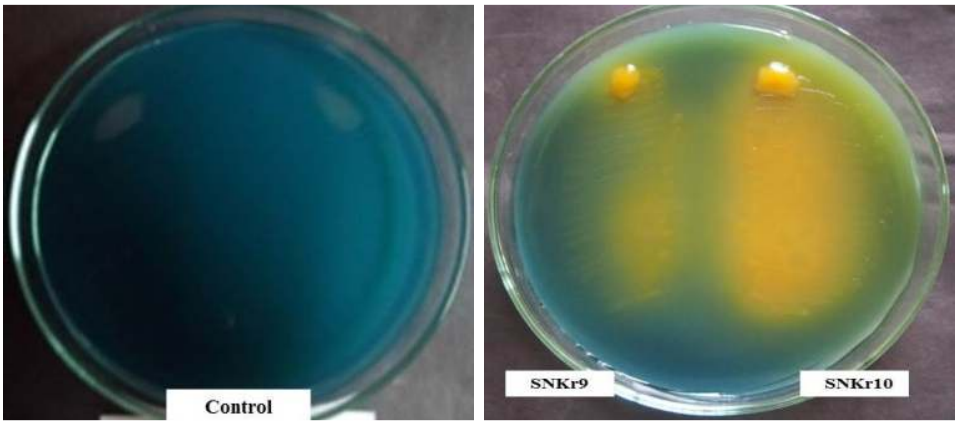


Fig. 9. *In vitro* BNF shown by SNKr10 on Jenson agar media

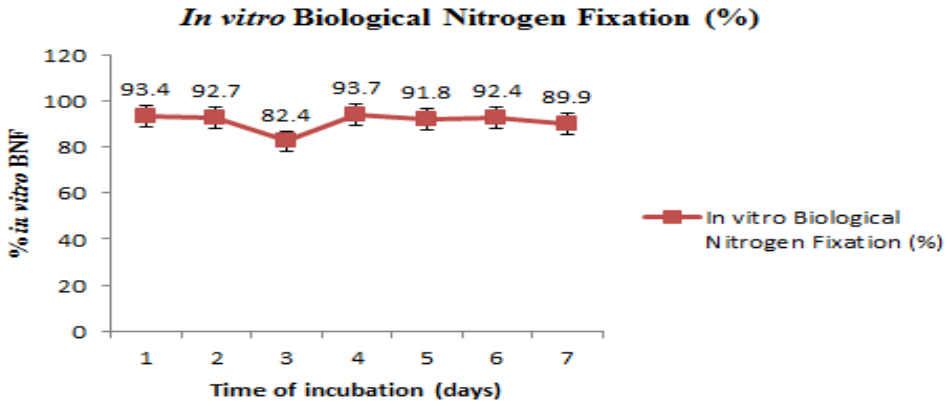
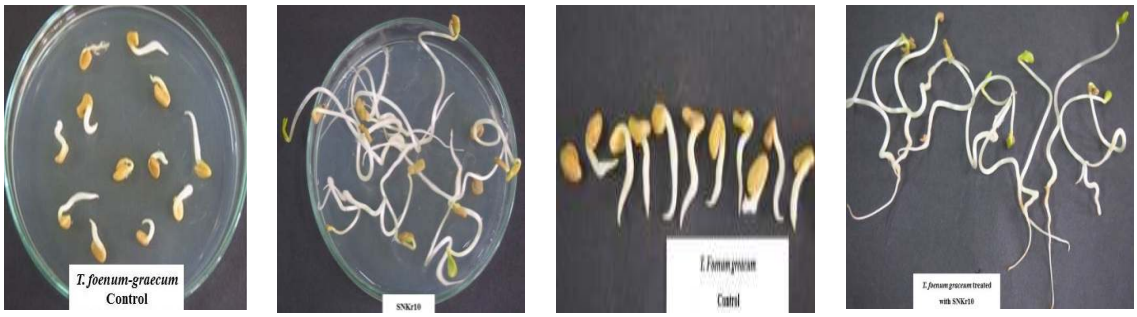


Fig. 10. *In vitro* BNF production by SNkr10 with time incubation

Table 5. *In vitro* BNF with sucrose concentration

Concentration of sucrose (%)	*In vitro* biological nitrogen fixation (%) (Mean ± S.D)
2	58.9±0.00
4	61.02±0.02
6	61.02±0.12
8	68.2±1.10
10	66.7±1.00



(a) **(b)**

Fig. 11. (a) *In vitro* Seed germination effect of SNKr10 on *T. foenum-graecum* Seedlings; (b). *In vitro* Seed germination effect of SNKr10 on *T. foenum-graecum* Seedlings

Table 6. Effect of selected bacterial isolates on the germination of *T. foenum-graecum*

Name of bacterial inoculum	Seed germination %	Seedling height (cm)	Root length (cm)	Shoot length (cm)	Wet Weight (g)	Dry weight (g)	Seedling vigor index
SNKr10	97.22 [A]	11.51±0.01	4.5±0.03	7.11±0.01	0.426±0.12	0.063±0.11	1121.91
Control	69.11	2.1±0.01	0.5±0.021	1.1±0.21	0.07±0.03	0.011±0.31	146.51

3.6 Pot House Experiment

In vivo seed germination parameters of *V. radiata* plants were studied by treating the plant seedlings with microbial inoculum of SNKr10 [Fig. 12 (a), 12 (b)]. We observed significant improvement in growth parameters of SNKr10 treated *V. radiata* plant in pot house as

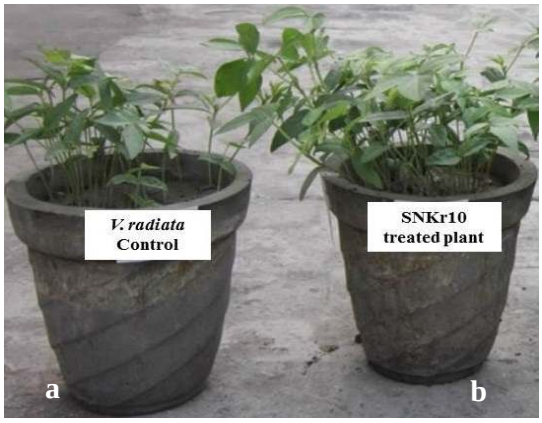


Fig. 12(a)

Fig. 12(b)

Fig. 12(a). Bacterization effect of consortium SNKr10 on *V. radiata* seedlings; a) control; b) SNKr10 treated plant

Fig. 12(b). Bacterization effect of consortium SNKr10 on the growth parameters of *V. radiata* seedlings; a) control; b) *V. radiata* plant treated with SNKr10

Table 7. Bacterization effect of SNKr10 on *V. radiata* seedlings

Growth parameters	SNKr10 treated seedlings	Untreated seedlings
% Seed germination	100	60
Seedling height (cm)	37.54±1.01	24.2±0.91
Root length (cm)	13.21±0.63	5.1±0.61
Shoot length (cm)	20.1 ±0.11	17.9±0.01
Wet root weight (g)	1.2±1.1	0.9±0.4
Wet shoot weight (g)	2.6±1.10	1.5±2.01
No. of pods	5±2.01	2±0.11
Dry root weight (g)	0.41±0.11	0.08±0.01
Dry shoot weight (g)	0.65±0.11	0.18±1.00
Total wet weight (g)	5.5±0.08	3.6±0.21
Total dry weight (g)	1.23±1.01	0.87±1.01
Vigor index	3754	1452

compared to control plants (Table 7). We found that SNKr10 treated plants showed 100% seed germination as compared to untreated plant (60%). Similarly seedling height; root/shoot length etc. (37.54 cm; 13.21 cm cm/20.1 cm) was also observed to be improved after treatment with SNKr10 as compared to control (24.2 cm; 5.1 cm/17.9 cm). The earlier study of Ali et al. [41] favoured our result outcomes by reporting the bacterization treatment of *Bacillus* sp. NpR-1 and *Pseudomonas* sp. AvH-4 improve the shoot length (11·30%) and fresh weight (40%) in comparison to control.

4. CONCLUSION

During present investigation it was observed that *B. thuringiensis* strain SNKr10 showed important role in plant growth promotion by exhibiting significant PGP attributes. Therefore this bacterial strain can be used as potent bio-inoculant for agriculture practises.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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