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Bacillus thuringiensis SNKr10, As Bio-Control Agent

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Abstract: ***Phyllosphere is the leaf surface of plants used as habitat for microorganisms. B. thuringiensis SNKr10 is a gram positive, rod shaped spinach phyllospheric bacteria (accession no. KU569966). This bacterium behaves as PGP bacteria by showing various PGP properties like IAA, siderophore, NH_3, HCN production etc. It also showed inhibition effect against plant pathogenic fungi (F. oxysporum, A. alternata, R. solani etc.). We also observed its positive effect on the growth parameters of S. oleracea using pot house experiment. Due to these properties of SNKr10, we can say that this bacterial is a potent bio-control agent and can be used as bio-fertilizer in sustainable agriculture.***

Keywords: Antimicrobial activity, Biocontrol agent, GC-MS, IAA, Siderophore

1. Introduction

Phyllosphere involves the total above-ground surfaces (leaves, stem flowers *etc.*) of a plant for microorganisms as habitat. The biocontrol agents are those PGPB that inhibit the various pathogens by the production of hydrolytic enzymes, antibiosis through inhibition of pathogen using hydrolytic enzymes or toxic compounds [1]. Plant growth promoting bacteria (PGPB) are the bacteria that improve plant growth by secretion of various useful regulatory chemicals like auxins, cytokinins and gibberellins; and biological nitrogen fixation *etc.*

Phyllospheric bacteria can promote plant growth by suppressing the infection of tissues caused by plant pathogens [2, 3] as biocontrol agents through the formation of secondary metabolites like siderophore, HCN and certain enzyme activity like chitinase, protease and peroxidase *etc.*, that showed toxic effect against plant pathogens *i.e.*, antifungal activity [4, 5, 6, 7].

Phyto-pathogen causes many plant diseases in plants and leads to lose of useful crops (aromatic, cultivable and medicinal plants) yield in agriculture [8]. These disease protection means of medicinal plants are still confined to the application of various chemical fertilizers as fungicidal form which is not much effective and also toxic for environmental point of view [9]. Therefore, biological control agents are gaining importance in the field of disease management of medicinal plants [10, 11]. The bioactive compounds are such biologically active substances that help in various reactions like inhibition of pathogenic microbes after forming as secondary metabolic products of some other useful microorganisms. In medical term, these are substances having an effect on the living tissues in positive or negative term that depends on the dose or the bioavailability of that compound [12]. These compounds can be usefully effective in wide range, like helps in good maintenance of health, and healing effect also [13].

The *S. oleracea* is a medicinally important bony plant that has various nutritional values. Various *Bacillus* sp. has reported earlier by various scientists that showed their biocontrol role against plant pathogens like *B. subtilis* OH 131.1 that inhibits fusarium head blight of wheat, *B. amyloliquefaciens* **inhibit fungal infection of maize plants and *B. subtilis* strain QST713 inhibit *P. striiformis* that caused yellow rust of wheat etc. [14, 15, 16]. *B. thuringiensis* SNKr10 is one of such type of non-pathogenic gram positive, endospore forming rod shaped and bacterium that was isolated from spinach phyllosphere. This bacterium plays an important role in agriculture through the inhibition of plant pathogenic microbes by producing antimicrobial compounds as secondary metabolic product. We also check the role of this bacterial strain on the plant growth using pot house experiment.**

2. Material and Methods

2.1 Sample collection and Isolation

Spinach leaves were collected from different fields of Haryana and Punjab (Chika, Karnal, Kurukshetra and Shahabad; Kapiyal and Sangroor), India. Fresh spinach leaves were surface sterilized with sterile distilled water and stored in sterile propylene bags and vortexed for 10-20 min. Serial dilutions in the range of 10^{-1}-10^{-5} were prepared and 100 µL of each dilution was spreaded on nutrient agar plates and incubated at 35-37 ^{o}C for 24-48 h and then preserved in nutrient slants for the study of bio-control activities [17].

2.2. Characterization of Bacterial Isolate

The bacterial isolates were examined for their morphological and biochemical properties including size of colony, shape, elevation, surface *etc.*, according to the Bergey's manual of systematic bacteriology [18]. These were further examined using gram's and endospore staining methods [19]. The DNA of bacterial isolate was extracted by using Biopure™ kits (Bioaxis DNA Research Centre) and 16S rRNA gene from extracted DNA was amplified using by PCR. The F and R primers were used for the amplification of 16S rRNA gene as: F = AGAGTTTGATCHYGGYTYAG (where Y and H are mixed base pair *i.e.*, Y=C/T, H= A/C/T), R = ACGGCTACCTTGTTACGACTT

PCR conditions were applied as: 1 cycle: 94 °C for 5 min (Initial denaturing); 35 cycles: 94 °C for 60 sec (denaturing); 53 °C for 45 sec (annealing); 68 °C for 90 sec (extension) and 68 °C for 10 min (final extension).

Amplified PCR product was electrophoresed by using agarose gel 1% in TAE buffer and visualized by staining with ethidium bromide (EtBr). PCR product was then purified by using sodium acetate and 70% of ethanol and eluted from the gel. Forward and reverse sequencing reactions of PCR amplicon were carried out on ABI 3730XL sequencer and sequences were obtained. These assembled DNA sequences were then submitted to NCBI and the accession no. was received. The phylogenetic tree was also prepared by the neighbor-joining method [20] using the distance matrix from the alignment (MEGA version 4) [21].

2.3. Screening for Plant Growth Promoting Activities

The screening of isolated bacterial isolate for PGP properties were performed by examining ammonia production, phosphate solubilization, IAA production, HCN and *In vitro* BNF. The ammonia production was performed by inoculating the fresh bacterial culture in 10mL peptone water broth and incubated at 30-37 ºC for 48-72 h. Nessler's reagent (0.5mL) was then added and incubated at room temp. The appearance of brown colour showed positive ammonia producer. The faint yellow was observed as weak ammonia producer and strong ammonia producer were confirmed by formation of deep brown colour [19]. Phosphate solubilization ability was observed by streaking of bacterial isolate on pikovskaya's agar media containing bromothymol blue dye and incubated at 35-37 °C for 3-5 days [22]. The clear zone of solubilization indicated as positive results.

Similarly, IAA production was confirmed by inoculating the respective bacterial isolate in Luria broth having 0.1% of L-tryptophan and allowed to incubate at 35-37 ^{o}C for 48-72 h. This incubated culture broth was centrifuged at 10,000 rpm for 10 min and then two drops of o-phosphoric acid were added into 2mL of supernatant along with 4mL Salkowski reagent (50mL, 35% of perchloric acid, 1mL of 0.5m $FeCl_3$ solution). It was observed for the development of pink colour within 2 h at room temp. [23]. The HCN production by bacterial isolate 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 culture plates were sealed with parafilm and incubated at 35-37 ºC for 3-5 days. Development of orange/red colour indicated the positive test [24].

In vitro BNF was observed by streaking the bacterial culture on nitrogen free Jensen's media using bromothymol blue (BTB) stain as an indicator dye and incubated at 35-37 ºC for 24-48 h. Yellow coloured zone around the bacterial colonies indicated the positive *in vitro* BNF [25].

2.4. Screening for Bio-control activity

2.4.1. Siderophore Production

The assay for siderophore production was performed in Chrome Azurole's (CAS) agar medium. CAS agar plates were streaked with the respective bacterial isolate. The incubation was done at 35-37 °C for 3-5 days. Development of halo zone around the bacterial colonies indicated the positive result [26].

For quantitative estimation of siderophore production the bacterial isolate was grown in minimal basal broth medium at 35-37 ^{o}C for 7 days under shaking conditions (100 rpm). After 24 h the cell free filtrate (CFF) was obtained due to centrifugation at 3000 rpm for 15 min. About 0.5 mL of CFF was mixed with equal volume of CAS solution and sulfosalicylic acid (10 µL) was added as shuttling solution. It was observed for the development of colour at room temp. within 20 min. The intensity of colour as detected spectrophotometrically at 630 nm [26]. Siderophore production (%) was calculated by using following formula:
(%) Siderophore production = O.D. of reference – O.D. of cell free filtrate/O.D. of reference

2.4.2(a). Antifungal activity

The isolate was tested for antagonistic activity against a wide range of phyto-pathogens on potato dextrose agar (PDA) medium by dual-culture technique. The isolate was grown in nutrient broth medium at 37 ^{o}C in a rotary shaker (150 rpm) for 3, 5, 7, 14 and 21 days to check the maximum inhibition effect due to production of secondary metabolites. The culture broth was then centrifuged at 12000 rpm for 10 min. to obtain the cell-free supernatant. The PDA plates were spreaded with different plant pathogenic fungi and wells (5-7 mm diameter) were made into the PDA plates by using a sterile cork borer, 100 µL of clear bacterial supernatant was also loaded into each well for the detection of antagonistic activity against different pathogenic fungi and allowed to incubate at 25-28 ^{o}C for 3-5 days. The antifungal activity of the culture filtrate was evaluated by measuring the diameter of inhibition zones [27].

In-vitro antifungal activity was also observed by inoculating bacterial culture and fungal pathogen in same petri plate in which bacterial isolate was streaked horizontally at the one side of the petri plate producing 2 sectors and at other sector disk of fungal pathogen was kept. Inhibition of fungal growth by the bacterial isolate determined antifungal activity. This test is known as visual agar plate assay or disk agar method [26].

2.4.2.1. Enzyme production for antifungal activity

Certain enzyme activities that showed inhibition of pathogenic fungi either by toxin production or by degradation of cell wall were detected by specific enzyme activity confirmation based agar medium like for protease screening, the bacterial isolate was streaked on casein agar medium and incubated at 37 ^{o}C for 48 h. A zone of proteolysis was detected on the casein agar plates after flooded with bromocresol green reagent (BCG) [29]. The chitinase enzyme activity was confirmed by streaking the bacterial culture on chitin agar media. The clear zone was formed around the colony after incubation at 35-37 ^{o}C for 5-

7 days that was confirmed by flooding the plates with 0.1% (w/v) congo red dye for 15 min. and then washed with distilled water [30].

2.4.2.2. Isolation and Characterization of Antimicrobial Compounds

2.4.2.2.1. Isolation of antimicrobial compound

The bacterial strain was grown in nutrient broth medium and incubated at 37 ^{o}C for 21 days. The filtrate was separated by centrifuging the inoculum containing broth at 10,000 rpm for 10 min. and then with the ethyl acetate in separating funnel (ratio of 1:1 v/v) followed by continues shaking for 30 min. This process was repeated thrice and the ethyl acetate solvent was allowed to evaporate under vacuum conditions. The concentrated crude extract was then used for separation using TLC [31].

2.4.2.2.2. TLC profile of the secondary metabolites

Thin layer chromatography (TLC) of the solvent extract was performed on a pre-coated silica gel TLC plate saturated with ethyl acetate: chloroform: acetic acid in the ratio of 50:40:10 as the solvent system. Ethyl acetate extract was spotted on the silica gel plate using capillary tube and placed in the TLC chamber containing the solvent and was appropriated to operate for about 9 cm. The chromatogram process was performed in closed glass tanks, in which the air has been saturated with elutant vapour by wetting a filter paper lining. The chromatogram was visualized under UV light at the range of 365 nm or through iodine vapours [31]. The Rf values of the compounds were calculated using the following formula:

Rf = Distance travelled by the compound/Distance travelled by the solvent front

2.5. Pot House Experiment

Pot house experiment was performed by treating the 15-20 surface sterilized *S. oleracea* seeds with 24 h old selected bacterial isolate (10^8 CFU mL^{-1}) for 1 h. The seeds were sown into pots filled with sterilized sandy loamy soil. The various growth parameters were recorded/calculated upto 14 days after bacterial treatment. During the experiment, the pots containing treated and un-treated seeds were irrigated with sterilized water every day [32, 33]. Various parameters such as germination efficiency, seedling height, root/shoot length, wet/dry weight, % seed germination and vigor index were calculated by the formula shown as:

Seed Germination (%) = No. of seeds germinated × 100/Total no. of seeds

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

2.6. Statistical analysis

Statistical analysis of needful data representation was done using SPSS version 16.0 lnc.

Results and Discussion

3.1. Morphological and Biochemical Characteristics

The SNKr10 bacterial isolate was isolated from *S. oleracea* phyllosphere. SNKr10 was observed as gram positive rod shaped, endospore forming, irregular in shape, dispersed elevation, with flat surface and creamish in coloured bacterial colonies. The SNKr10 was also found to be positive for nitrate reduction, starch hydrolysis, voges prausker (VP), citrate utilization *etc.*

3.2. Screening for PGP activities

It was observed that SNKr10 bacterial isolate exhibit significant PGP properties like phosphate solubilization having solubilized halo zone of 1.8 cm. Similarly SNKr10 showed positive reaction for ammonia, phosphate solubilization, IAA (34 µg/mL after 3 days of incubation), *in vitro* BNF and HCN production *etc.* [34]. Verma et al. [35] also favoured our findings by reporting the role various methylotrophic bacteria as PGPB, isolated from wheat (*T. aestivum*) phyllosphere by production of IAA, ammonia production and phosphate solubilization *etc.*

3.3. Result of Screening for Bio-control activity

3.3.1. Siderophore Production

Siderophore production by SNKr10 was confirmed by formation of yellow to orange halo on CAS agar plates *i.e.*, 25 mm [Fig. 1 (a)]. Siderophore is an iron relating promoter that are produced by bacteria and some fungi in iron limited area that may help to provide iron molecules to plant for growth from the deep region of soil. It plays an important role in transfer of electrons; provide resistance to active oxygen intermediates and RNA synthesis. Beside this, it also helps in protection of plants against pathogenic microorganisms as bio-control agent by preventing iron for plant pathogens [36].

Deb et al. [37] also reported that maximum of 16 mm of CAS halo zone was formed by isolate KD7 on CAS agar plates and therefore favour our findings. Similarly, Verma et al. [38] also reported that *Alcaligenes faecalis* IARI-NIAW1-6 and *Rhodobacter capsulatus* IARI-NIAW1-9 (Wheat associated PGPB) had shown the ability to produce maximum of 4.9 mm and 4.6 mm of halo zone respectively.

Quantitatively, it was observed that significant SU (78.8% after 8 days of incubation) was produced by SNKr10 after 8 days of incubation (Fig. 1(b); Table 1). The reports of Sharma et al. [39] reported that *P. fluorescence* strain An-14-kul isolated from apple rhizosphere are also positive for siderophore production and showed 67.27% S.U in liquid assay. Cherif-Silini et al. [40] reported that *Bacillus* strain B25 isolated from wheat rhizosphere produced maximum of 53.05% siderophore followed by BA11 (51.51%) and D11 (43.5%) and therefore favour our findings.

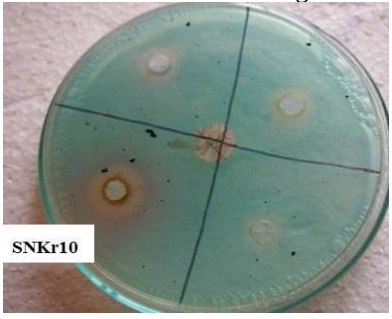

Figure 1(a)

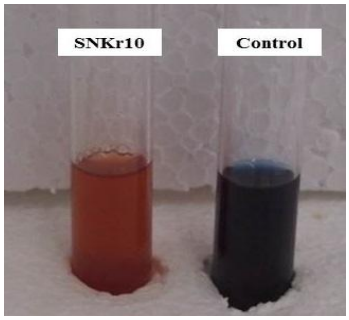

Figure 1(b)

Figure 1: Siderophore production by SNKr10; a) Siderophore production shown by SNKr10 on Cas Agar plate; b) Cas broth

Table 1: % Siderophore production by SNKr10 with time incubation

Incubation time (days)	Percentage Siderophore units (% SU) (Mean ± S.D)
1	33.3±0.02
2	41.9±0.12
3	50±0.01
4	62.1±1.00
5	68.7±0.00
6	70.7±0.01
7	78.3±0.05
8	78.8±0.21
9	75.2±1.22
10	77.3±1.00

3.3.2(a). *In vitro* Antimicrobial activity

Antifungal activity was identified through clear zone formation around bacterial colonies. The significant inhibition was shown by SNKr10 against five plant phytopathogenic fungi namely against *F. oxysporum*, *A. alternata*, *R. solani*, *Cladosporium* sp. and *Curvularia* sp. after 21 days of incubation (Table 2; Fig. 2.1; Fig. 2.2; Fig. 2.3; Fig. 2.4). Ji et al. [41] supported our result outcomes by reporting that endophytic diazotrophic bacteria like *Microbacterium* sp. strain SW521-L21, *Klebsiella* sp. strain KW7-S06 and *Bacillus* sp. strain CB-R05 isolated from Korean rice plants shows the maximum antifungal activity against *F. oxysporum* and *R. solani* after 7 and 14 days of incubation at 28 ^{o}C. Deng et al. [42] also reported in favour of our findings by reporting the antimicrobial activity of *Burkholderia contaminans* MS14 against plant pathogens due to development of biopesticides and pharmaceutical drugs and therefore behaving as PGPB as well as biocontrol agents.

Similar inhibition effect was observed along with varying conc. of bacterial inoculum *i.e.*, 200-2000 µg mL^{-1} ethyl acetate extract of SNKr10 in DMSO that showed (at 2000 µg mL^{-1} of bacterial CFF conc.) (Table 3). It was also observed that SNKr10 showed significant % inhibition effect against these plant pathogenic fungi (Fig. 3).

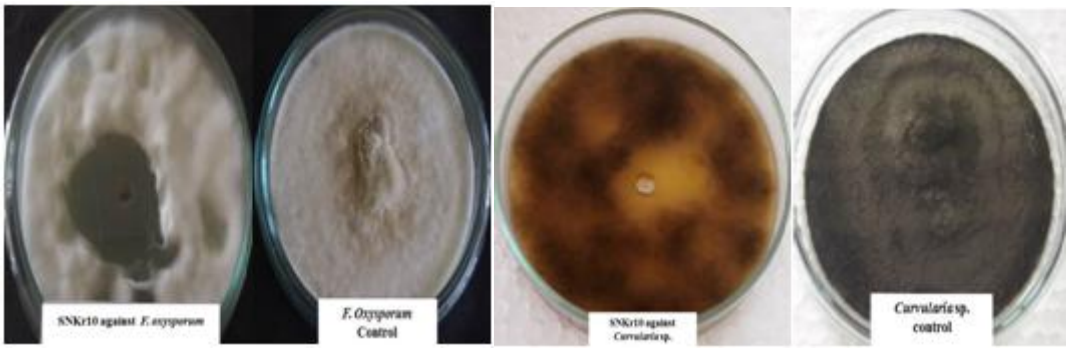

Figure 2.1: Antifungal activity shown by SNKr10 against *F. oxysporum* in comparison to control

Figure 2.2: Antifungal activity shown by SNKr10 against *Curvularia* sp.

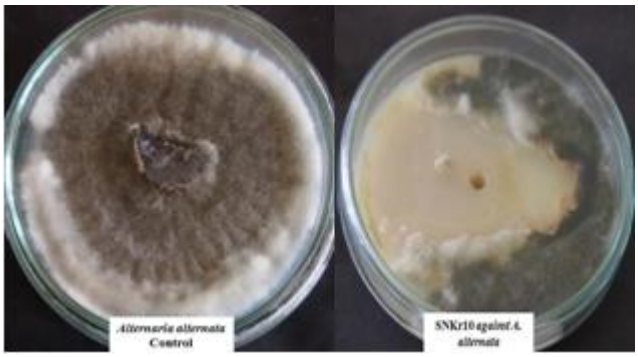

Figure 2.3: Antifungal activity shown by SNKr10 against *A. alternata*

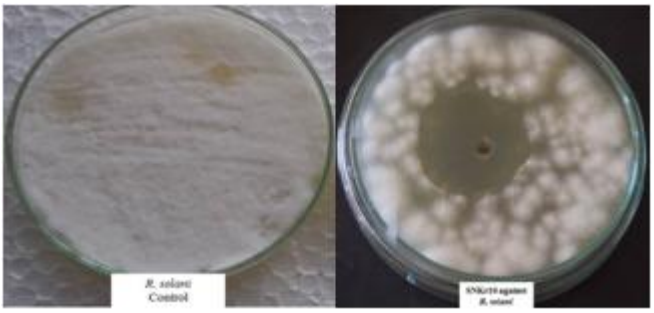

Figure 2.4: Antifungal activity shown by SNKr10 against *R. solani*

Table 2: Effect of SNKr10 against plant pathogens

Incubation time (days)	Effect of SNKr10 against Plant pathogens as inhibition zone (Mean ± S.D)				
	R. solani (mm)	*F. oxysporum* (mm)	*A. alternata* (mm)	*Cladosporium* sp. (mm)	*Curvularia* sp. (mm)
3	3±0.00	0±0.00	0±0.00	0±0.00	0±0.00
5	0.2±0.00	0.9±0.00	0.3±0.00	0.4±0.00	0.2±0.00
7	0.8±0.01	11.1±0.91	10.9±0.00	10.3±0.04	10±0.09
14	12±0.04	16.9±0.09	13.4±0.25	13.2±0.32	13.24±0.23
21	23.25±0.40	21.2±0.11	23.7±0.07	15±0.10	14.92±0.40

Table 3: Effect of SNKr10 bacterial inoculum conc. on plant pathogenic fungi

SNKr10 bacterial inoculum (µg/mL)	*R. solani* (mm ± SD)	*F. oxysporum* (mm ± SD)	*A. alternata* (mm ± SD)	*Cladosporium* sp. (mm ± SD)	*Curvularia* sp. (mm ± SD)
200	7.04±0.03	8.21±0.01	9.34±0.01	7.80±1.01	7.09±0.21
400	8.28±0.11	8.99±0.01	9.51±0.13	8.89±1.00	8.51±0.10
600	10.09±0.10	10.77±0.21	10.98±0.31	10.25±0.03	10.1±0.20
800	11.53±0.21	14.67±0.13	13.2±0.10	12.8±0.02	12.0±0.01
1000	13.31±0.02	17.54±0.03	16.5±0.14	13.9±0.10	12.95±0.11
1500	15.98±0.01	20.11±0.01	19.3±0.02	16.62±0.01	16.04±0.01
2000	23.91±0.03	22.2±0.01	24.8±0.02	19.88±0.02	18.71±0.13

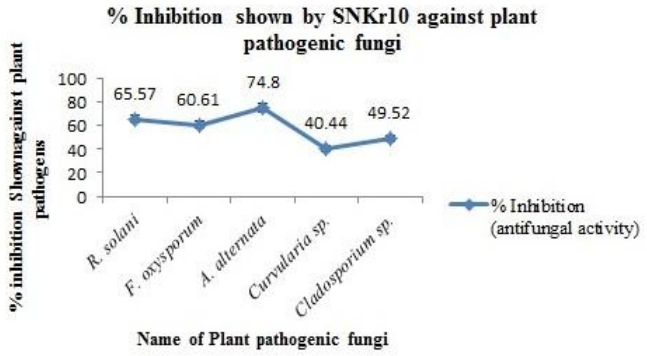

Figure 3: Percentage inhibition by SNKr10 against plant pathogenic fungi

3.3.2.1. Hydrolytic enzyme activity

SNKr10 bacterial isolate showed the properties of chitinase and protease enzyme activity that help to degrade the cell wall and protein structure of pathogenic fungi (Fig. 6; Fig. 7). Kuddus and Ahmad [41] also reported the antimicrobial activity is due to chitinase enzyme and therefore favour our findings. It has also been earlier reported that certain bacteria like *Bacillus* sp. and *Paenibacillus* sp. produces lipopeptides as secondary metabolites that help to activate the protease enzyme activity for the inhibition of pathogenic microbes [43] which also favours our findings.

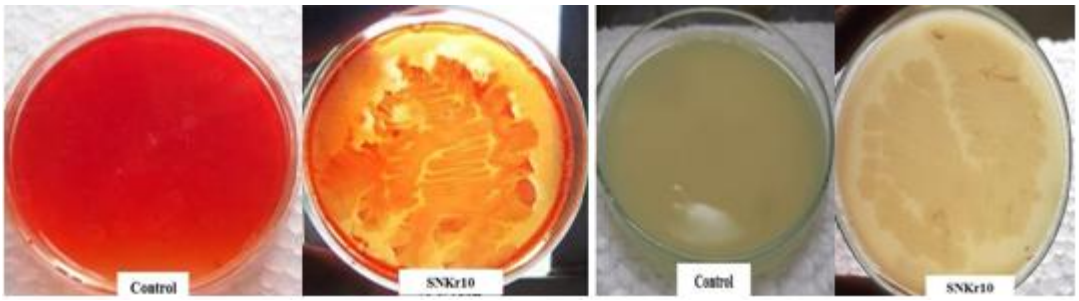

Figure 6: Chitinase enzyme activity shown by SNKr10 on chitin agar plate
Figure 7: Protease enzyme activity shown by SNKr10

3.3.2.2. Isolation and Characterization of antimicrobial compound

The antimicrobial compounds are usually secondary metabolites that were isolated or extracted from SNKr10. The extracted compounds were then characterized using TLC, Uv-Vis spectroscopy.

3.3.2.3. TLC and UV-Vis analysis

During TLC analysis, maximum solubility of extracted antimicrobial compounds was observed in solvent having ethylacetate:chloroform:acetic acid (50:40:10). We also observed the Rf value of SNKr10 and chlorophenicol, in which chlorophenicol was taken as positive control (Table 5). These Rf values of all the unknown extracted antimicrobial compounds revealed that their structural as well as functional group could be resembled with antibiotic like chloramphenicol. Their mobility as clear band was visualization using iodine vapours or under UV light [Fig. 8(a); (b)]. We also observed that their maximum stability was at temperature even above from 150-180 ^{o}C. This antimicrobial compound production from *Exiguobacterium mexicanum* using crude extraction method (ECA) [31]. The earlier literature study also involves the antimicrobial compounds extracted from useful plants in the form of antibiotic was purified using TLC analysis [44, 45]. Attar et al. [46] also reported the purification of *Streptomyces lydicus* extracted antimicrobial compounds using TLC analysis and therefore favors our findings.

These unknown antimicrobial activity carrying compounds extracting from SNKr10 bacterial isolates were partially identified using UV-Vis spectrophotometer analysing by taking O.D. at variable range from 200-400 nm (Table 6). These data resembled with the peak of antimicrobial compounds (250-280 nm) as, Wammer et al. [47] reported the maximum peak of norfloxacin, enrofloxacin and ofloxacin antibiotics at 271 nm and 286 nm respectively using ultraviolet-visible diode array detector which is in accordance to our findings. Therefore the structural or functional group of SNKr10 bacterial compounds could be resembled with the various antimicrobial compounds.

Figure 8 (a): TLC analysis of SNKr10 extracted secondary metabolic compounds visualization using KI

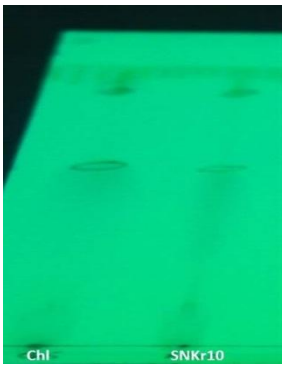

Figure 8 (b): TLC analysis of SNKr10 extracted secondary metabolic compounds visualization by keeping TLC plates under UV light

Table 5: TLC Analysis of SNKr10 Bacterial Isolates

Antimicrobial compound extraction from SNKr10	Total distance covered by unknown compound (cm)	Rf value
SNKr10	7; 4.6	0.87; 0.57
Chloramphenicol (positive control)	6.4; 4.4	0.8; 0.55

Table 6: UV-Vis spectroscopy study of unknown compounds extracted from SNKr10

Wavelength (nm)	UV-Vis spectrophotometer (200-400 nm λ) of SNKr10 O. D. value (Mean ± SD)
200	0.054±0.01
220	0.056±0.05
240	0.062±0.01
260	0.420±0.10
280	0.942±0.11
300	1.025±0.20
320	1.024±0.05
340	1.025±0.03
360	1.011±0.11
380	0.923±0.01
400	0.055±0.01
420	0.67±0.21

3.3. Molecular Characterization of SNKr10

It was observed that bacterial isolate SNKr10 showed 100% identity to the sequence of *Bacillus thuringiensis*. The sequences were submitted to the NCBI and received the accession no. *i.e.*, KU569966. The phylogenetic tree was also constructed using sequence (Fig. 13).

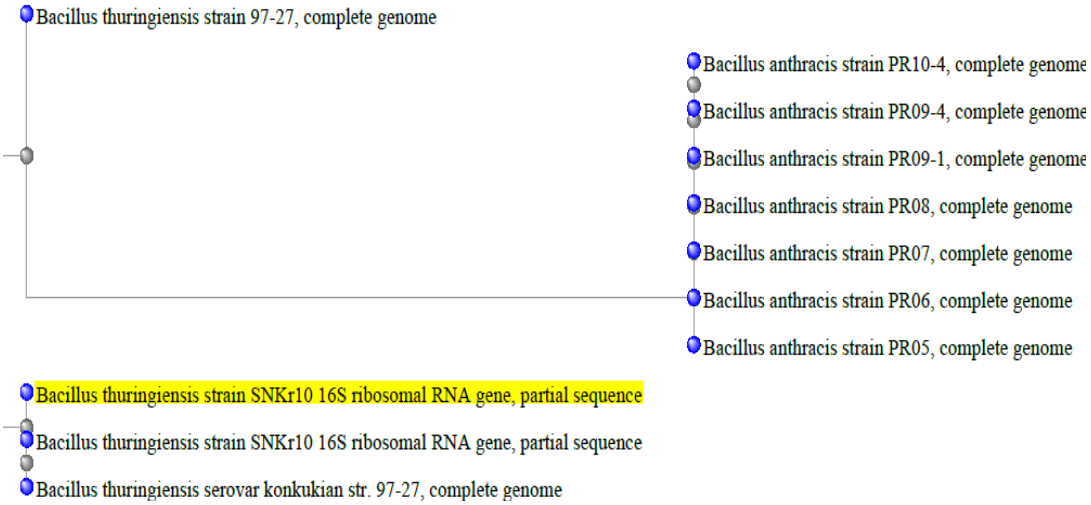

Figure 13: Phylogenetic Tree of *Bacillus thuringiensis* SNKr10

3.4. Pot house experiment

We observed significant improvement in growth parameters of SNKr10 treated *S. oleracea* seedlings in pot house in comparison to untreated control (Fig. 14(a), Fig. 14(b); Table 9). The earlier study of Ali et al. [48] 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. The SNKr10 bacterial treatment also improved the shoot, root length *etc.* of *S. oleracea* plants in comparison to untreated plant. Ahirwar et al. [49] also supported our result outcomes by reporting the *P. fluorescence* strain SS5 role in increasing the growth and biomass (root and shoot weight/length, fruit yield per plant, and total fruit yield) of tomato plants in comparison to control.

Figure 14 (a), Figure 14 (b)

Figure 14 (a): Bacterization Effect of SNKr10 on *S. oleracea* Seedling using pot house experiment; a) control; b) SNKr10 treated plant

Figure 14 (b): Effect of SNKr10 inoculum on the growth rate of *S. oleracea* seedling in comparison to untreated plant; a) control; b) SNKr10 treated plant

Table 9: Bacterization Effect of SNKr10 on *S. oleracea*

Growth Parameters	SNKr10	Control
% Seed germination	99.4	75
Seedling height (cm)	20.03±0.01	16.4±0.11
Root length (cm)	13.02±0.03	5.2±0.01
Leaf length (cm)	11.12±0.01	8.2±1.01
No. of leaves	20.7±1.15	9.2±0.01
Total wet weight (g)	6.1±0.20	3.1±0.11
Total dry weight (g)	0.84±0.001	0.35±1.00
Vigor index	1990.98	1230

Mean ± Standard deviation; *All values are ($P \leq 0.05$) significant as compared to control; Same letters are not significantly different at 0.05 levels

3.5 Conclusion

During present study, it was observed that *B. thuringiensis* SNKr10 showed significant role as bio-control agent. As it showed important role in inhibition of plant pathogenic microorganisms by producing HCN, siderophore, antifungal activity *etc.* Therefore this bacterial strain can be used as important bio-control agent for the sustainability of agriculture.

3.6 Conflict of interest

Author has no conflict of interest

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