KAVAKA 61(3): 48-54 (2025) DOI: 10.36460/Kavaka/61/3/2025/48-54

Isolation and Characterization of *Streptomyces alkaliphilus* from Thar Desert Rhizosphere with Plant Growth-Promoting and Antifungal Activities

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(Submitted on May 27, 2025; Accepted on July 12, 2025)

ABSTRACT

The Indian Thar Desert presents a harsh environment characterized by extreme conditions such as low moisture in sandy soils, high salinity and elevated soil temperatures, which collectively limit its biodiversity. In the present study, actinobacteria were isolated from the soil of the Bikaner region in the Thar Desert and their potential as plant growth-promoting and antifungal agents was evaluated. A strain identified as *Streptomyces alkaliphilus* BKA-16 was isolated from the rhizosphere of the native plant *Prosopis cineraria* and identified through 16S rDNA sequencing. Strain BKA-16 demonstrated notable antifungal activity against *Colletotrichum truncatum*. Furthermore, it showed the ability to produce phytohormones, including indole acetic acid and ammonia production, and exhibited a clear zone indicating phosphate solubilization. These findings suggest that *S. alkaliphilus* BKA-16, isolated from the arid Thar Desert, holds promise as a plant growth-promoting bacterium and a biocontrol agent capable of suppressing *C. truncatum*, the causal organism of anthracnose in green gram, within arid ecosystems of India.

Keywords: Indian Thar Desert, rhizosphere, *Streptomyces alkaliphilus, Prosopis cineraria,* Plant Growth Promotion properties

INTRODUCTION

The Great Indian Thar Desert is the 9th largest subtropical desert, characterized by a wide range of arid to subhumid climatic conditions (Masand *et al*., 2018). It presents a unique and extreme habitat due to its harsh environmental features, including high summer temperatures, cold winters, sandy soils with limited water retention and elevated salinity levels in both soil and water (Neelam *et al*., 2018). These extreme conditions make desert ecosystems relatively unexplored in terms of microbial diversity, particularly microorganisms with potential plant growth-promoting capabilities (Tiwari and Gupta, 2013).

Given this, it is suggested to investigate either rare genera from conventional habitats or less-explored species thriving in extreme environments such as deserts (Harwani, 2013; Fatahi-Bafghi *et al*., 2019). Recent research has increasingly focused on actinobacteria from desert soils, motivated by their potential to produce novel antimicrobial compounds (Almuhayawi *et al.,* 2021). Within the rhizosphere of desert plants, actinomycetes especially those belonging to the genera *Streptomyces*, *Micromonospora* and *Nocardia* are commonly found and may occur in substantial numbers (Xu *et al*., 2015).

Among these, the genus *Streptomyces* is the most abundant and well-studied, known for its significant biocontrol potential against phytopathogenic fungi and its role as a biofertilizer (Olanrewaju *et al*., 2019; Wang *et al*., 2020). Although *Streptomyces* species are widely distributed in soil environments, they can also establish endophytic relationships within plant tissues such as roots, leaves, and stems (Van der *et al*., 2018). These microbes are notable producers of natural bioactive compounds, contributing to nearly two-thirds of known antibiotics and are recognized for their role in promoting plant growth and suppressing plant pathogens (Sharma *et al*., 2018). Considering these factors, the present study aimed to evaluate the antifungal activity and plant growth-promoting traits of the actinobacterium *Streptomyces alkaliphilus*, isolated from the rhizosphere soil of the native desert tree *Prosopis cineraria*.

MATERIALS AND METHODS

Isolation of *Streptomyces alkaliphilus* strain BKA-16

Strain BKA-16 was isolated from rhizospheric soil adhering to the roots of the native plant *Prosopis*

cineraria in Bikaner, Rajasthan, India. One gram of soil was suspended in 9 mL of sterile distilled water and thoroughly mixed by shaking at 150 rpm for 10 minutes to create a homogenized stock solution. This suspension served as the base for serial dilutions using sterile distilled water. To isolate *Streptomyces* spp., 1 mL aliquots from each dilution were plated using the pour plate technique on KenKnight and Munaier (K&M) agar medium. The inoculated plates were incubated at 30±2°C for six days. Colonies with characteristic morphology were selected and subcultured on the same medium for purification. The purified strain, designated BKA-16, was preserved on K&M agar slants at 4°C for subsequent analysis. Finally, the strain BKA-16 was stored on KenKnight & Munaier agar slant at 4°C for further investigations.

Genomic characterization of the strain BKA-16

Strain BKA-16 was grown at 28±2°C for 7 days in a flask containing 100 mL KenKnight & Munaier broth medium and incubated in mechanical shaker cum incubator. Genomic DNA was extracted with Qiagen-50 Fungal/Bacterial DNA isolation Kit according to the manufacturer recommendations. PCR amplification of the 16 S rDNA of the strain was performed using two primers: 27F (5′-AGAGTTTGATCCTGGCTCAG-3′) and 1492 R (5′-GGTTACCTTGTTACGAC TT-3′) (Heuer *et al*., 1997). The 16 S rDNA gene sequence was amplified using final volume of reaction mixture 50 µL. The PCR amplification was performed using the DNA thermal cycler program as follows: 94°C for 2 min (initial denaturation), 94°C for 1 min (denaturation), 54°C for 1.5 min (annealing) and 72°C for 1 min (extension). The amplification procedure was continued for 35 cycles and terminated with a final step at 72°C for 15 min. The PCR product was detected and visualized using 1% agarose gel electrophoresis. PCR product was purified and sequenced. The nucleotide sequences were compared to online databases at the National Centre for Biotechnology Information (NCBI) website (www.ncbi.nlm.nlh. gov) using the BLAST program. Phylogenetic tree was constructed through the neighbor-joining (NJ) method using MEGA11 software.

IAA production by strain BKA-16

Pure culture of the strain BKA-16 was inoculated in nutrient broth and incubated for 24 hrs at 30±2°C. Supernatant solution (100 µ) of the pure cultures was inoculated in Ashbys Mannitol broth containing 1% L-Tryptophan and incubated at 30±2 °C for 48 hrs in the dark followed by determination of IAA in supernatant. Development of pink colour was considered to be positive on addition of Salkowski reagent. The absorbance was read at 530 nm in a double beam UV– Visible spectrophotometer. A standard curve (10–100 µg/mL of IAA) was plotted for quantification of IAA solution. Un-inoculated medium with a reagent was used as a blank. The amount of IAA in the culture supernatant was expressed as µg/mL.

Phosphate solubilization by strain BKA-16

The culture of strain BKA-16 was spotted on Pikovskaya agar plates to determine the phosphate solubilization ability of isolated strain (Pikovskaya, 1948). The plates were incubated at 28±1°C for three days and observed for colonies forming halo zones.

Ammonia production by strain BKA-16

Ammonia production was done by method given by Thomas (1912). The culture of strain BKA-16 was grown in the peptone water in test tubes and incubated for 4 days at 30±1°C. After 4 days, 1 ml of Nesseler reagent was added in each tube. Presence of a faint yellow colour indicated small amount of ammonia and deep yellow to brownish colour indicated the maximum production of ammonia. The quantification was done based on Nesslerization spectrophotometric assay given by Goswami *et al*., 2017. 0.5 mL supernatant of strain BKA-16 was mixed with 1 mL of Nessler's reagent in test tubes separately previously washed with ultrapure water and incubate at room temperature (27±2°C) for 10 min then diluted 6x to made up the final volume to 9 ml and measured at 450 nm by using UV- Visible Double Beam spectrophotometer (Model No. RG-101-08). The standard curve of ammonium sulphate $(NH_4)_2SO_4$ at different concentrations (500M to 4000M) was used.

Antifungal activity of the strain BKA-16 in *in-vitro*

In vitro evaluation of the antifungal potential of the strain BKA-16 against *Colletotrichum truncatum* was conducted by the dual culture method Barhate *et al.* (2012). Both the strain and *C. truncatum* were co-cultured on the 9 cm Petri dish containing PDA medium. Mycelial fungal discs (5 mm diameter) of 7 days old culture of *C. truncatum* (taken from the edge of an actively growing fungal colony) were placed separately in the center of PDA plates. And the strain BKA-16 was streaked parallelly to each other 1 cm away from the disc on both sides in the same plate thrice replicated. The plate with only fungal disc served as control. The plates were incubated at

28±1°C till the control plates attained full growth. Inhibition percentage were calculated using the formula described by Vincent (1947).

$$Inhibition\ (\%) = \frac{C - T}{C} * 100$$

Where, C = radial growth of *C. truncatum* in control (cm) T = radial growth of *C. truncatum* in strain BKA-16 (cm).

Statistical analysis

Data were statistically processed and subjected to analysis of variance (ANOVA) using software SPSS Statistics version 16. Significant differences among treatments were based on the Tukey's multiple range test at $P < 0{\cdot}05$. Results are presented as means of three replicates ± standard deviation.

RESULTS

Identification of strain BKA-16 based on 16 S rDNA gene sequences analysis

Streptomyces associated with rhizosphere of *P. cineraria* was isolated and identified to genus and specie levels based on 16 S rDNA gene sequences analysis. The 16 S rDNA sequence (436 nucleotides) of the strain BKA-16 was determined and has been deposited with the GenBank data library under the accession number PV624827. The sequence of strain BKA-16 was aligned with those of *Streptomyces* reference species available in the GenBank database, which confirmed that the strain BKA-16 belonged to the genus *Streptomyces.* Its position of strain BKA-16 in the 16 S rDNA *Streptomyces* tree was illustrated in **Figure 1**. The primary comparison of 16 S rDNA sequence with reference strains using BLAST indicated that BKA-16 showed 98.61% identity with the most closely related species such as *Streptomyces alkaliphilus* strain IF17 (MH425398) and *Streptomyces alkaliphilus* strain SL26 (MT669319). However, strains showing a 16 S rDNA sequence identity of 98.61% do not all belong to the same species. In the fact, some representatives of validly described *Streptomyces* species, such as the type strains of *Streptomyces calidiresistens* QT195 (MT072148) showed sequence identity of 98.61%.

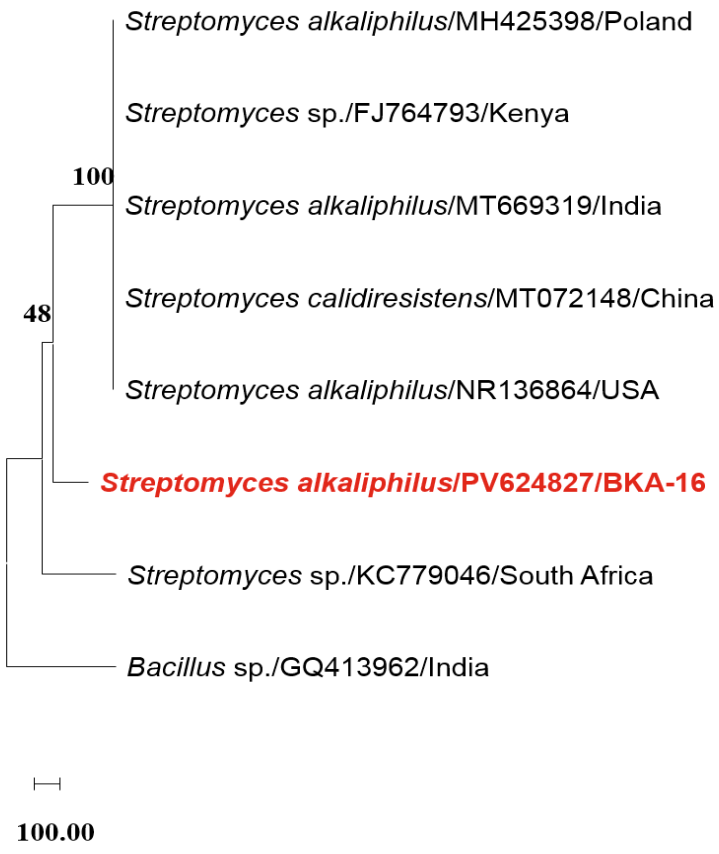

Figure 1: Neighbor-joining phylogenetic tree based on 16 S rDNA gene sequences showing relationship of strain BKA-16 with its closely related *Streptomyces* strains.

IAA production

Streptomyces alkaliphilus strain BKA-16 isolated from rhizosphere of *P. cineraria* was evaluated for their ability to produce IAA, a phyto hormone essential for plant growth and development. BKA-16 could produce significant amounts of IAA indicated by the appearance of pink colour in the nutrient broth. Data presented in **Figure 2** showed that IAA production by the strain BKA-16 ranged from 27.50 to 220.25 µg/mL in nutrient broth media amended with L- tryptophan. On the other hand, the results showed that the production of IAA by *Streptomyces alkaliphilus* strain BKA-16 was started from 1st and reach to maximum quantity at 4th day of incubation then gradually decreased.

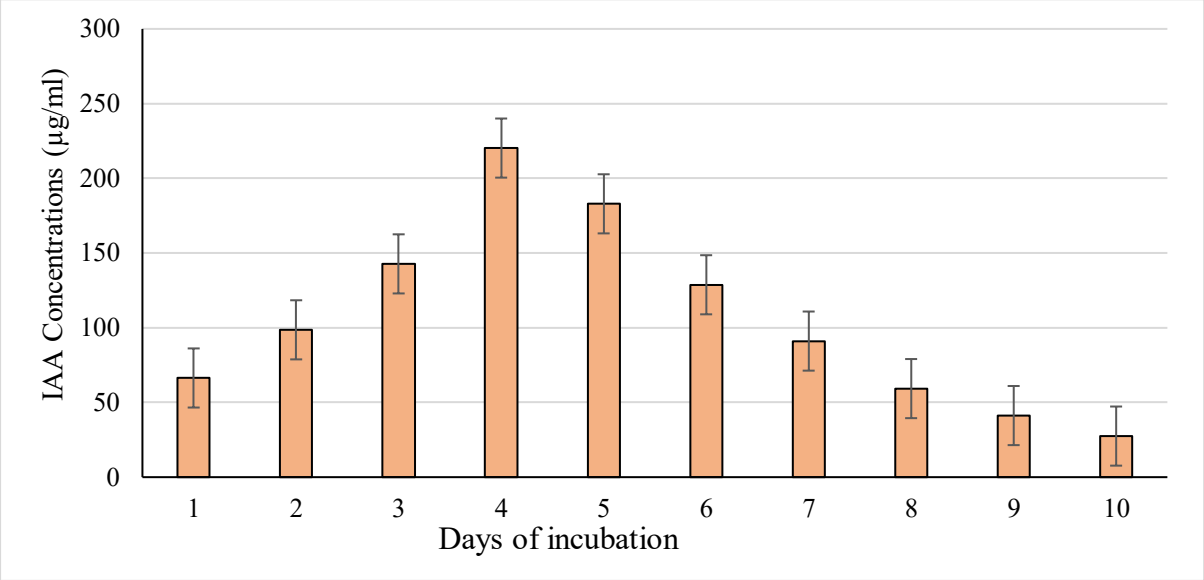

Figure 2: Indole-3-acetic acid (IAA) production by *Streptomyces alkaliphilus* strain BKA-16

Phosphate solubilization

It was noticed that strain BKA-16 could form clear halo zone (18.23 mm) around the spot inoculated in Pikovskaya media, indicating the ability to solubilize phosphate.

Ammonia production

It was observed that strain BKA-16 produced ammonia in peptone water. Maximum production of ammonia was 1.75 µmol ml^{-1} after 72 hrs of incubation at 30±2°C with constant agitation. However, ammonia produced in the respective growth medium did not increase after 72 hrs of incubation (**Figure 3**).

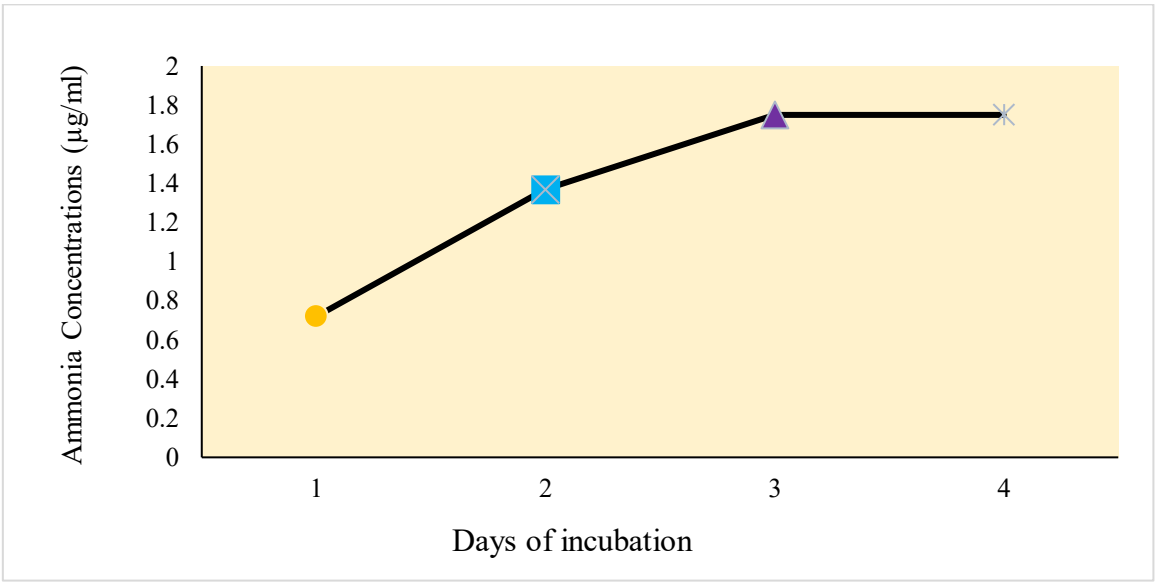

Figure 3: Ammonia production by *Streptomyces alkaliphilus* strain BKA-16

Antifungal activity of the strain BKA-16

The strain BKA-16 was screened for antagonistic activity against tested pathogen, *Colletotrichum truncatum*. *Streptomyces alkaliphilus* strain BKA-16 exhibited significant antagonistic activity against tested pathogen in the dual culture plate assay as shown in **Figure 4**. This result indicated that the per cent mycelial inhibition of 50.16% as comparison with control.

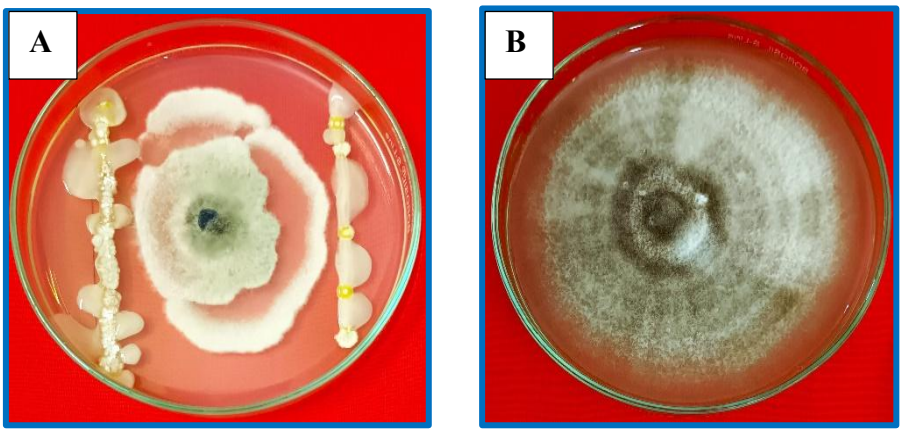

Figure 4: Mycelial growth inhibition of *C. truncatum* by *Streptomyces alkaliphilus* strain BKA-16 (A) and control (B).

DISCUSSON

Isolation source of *Streptomyces alkaliphilus* strain BKA-16 was *P. cineraria*'s rhizosphere, a plant belonging to Indian Thar Desert of Bikaner, Rajasthan. This is the unique saline desert which contains high sand content (Gupta and Ansari, 2014). To characterize any rhizobacteria as PGPR it should be attributed to the production of diverse metabolites including IAA, siderophores, ammonia, and activities such as phosphate solubilization (El-Deeb *et al*., 2012). The phytohormone, indole-3-acetic acid (IAA), plays a major role in plant development and its supply is capable of supporting its host during biotic stress condition like pathogenic attacks (Sathya *et al.,* 2017). Our results showed that the IAA production by *Streptomyces alkaliphilus* strain BKA-16 was similar to reported *by* Abdelmoteleb and Gonz´alez-Mendoza (2020); Xu *et al*. (2025) who reported that IAA producing *Streptomyces* sp. MBRL 10 and *Streptomyces netropsis* strain A-ICA promoted seed germination and rice seedlings growth. Solubilization of phosphate is an important mechanism of plant growth promotion (Moorthy *et al*., 2025). *Streptomyces* species are capable of increasing the availability of phosphorus (P) to plants through mechanisms such as the secretion of phosphatase to free P bound in organic matter and the production of organic acids / chelating substances that helps to decrease rhizosphere pH (Tamreihao *et al*., 2018). In this regard, the result from the present study conforms to other previous studies on phosphate solubilization. Ammonia production is another important trait of PGPR where an organism can break down complex nitrogenous materials like peptones and release ammonia in soil. Released ammonia is taken up by plant as a nutrient source. Soils which are rich in nitrogen, there can be an accumulation of ammonia creating alkaline condition of the soil which suppresses the growth of certain fungi (Uysal *et al*., 2025). In present study, ammonia produced by *Streptomyces alkaliphilus* strain BKA-16 is at parity with several PGPR strains including *Arthrobacter arilaitensis* and *Streptomyces pseudovenezuelae* (Chukwuneme *et al*., 2020).

The results of present investigation showed that the inhibitory effect of *Streptomyces alkaliphilus* strain BKA-16 against mycelial growth of *Colletotrichum truncatum* may be due to its ability to release antagonistic substances such as spreadable antibiotics, hydrolytic enzymes (cellulases, chitinase, and glucanase) and siderophore, when they interacted with the fungus evaluated (Colombo *et al.*, 2019). Similar results were observed for actinobacterial strains isolated from extreme conditions in the Saudi Arabian desert which showed antimicrobial activity against both gram-positive and gram-negative bacteria, as well as against fungi (Nithya *et al*., 2018). The results of Zhong *et al*. (2025) also in line with the results of present study who showed that *Streptomyces*

olivoreticuli ZZ-21 has antagonistic ability against *Colletotrichum scovillei* in both *in vitro* and *in vivo* due to strain ZZ-21 inhibited spore germination and damaged cell membrane permeability of *C. scovillei.*

CONCLUSION

The findings of present investigation highlighted that phosphate solubilization, antifungal activities, IAA and ammonia producing *Streptomyces alkaliphilus* strain BKA-16 from rhizospheric soil of Indian Thar desert of Bikaner region could be easily isolated and may be exploited after strain improvement for research. However, further studies using *Streptomyces alkaliphilus* strain BKA-16 are needed to explore the exact contribution of IAA, phosphate solubilization, ammonia production and antifungal activity in the promotion and protection of plant growth as well as their contribution in sustainable agricultural development.

ACKNOWLEDGEMENTS

This study was supported by the Swami Keshwanand Rajasthan Agricultural University, Bikaner, Rajasthan, India.

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