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Research Article

Antibacterial Potential Of *Bacillus* Sp. TBS Isolated From Tomato Rhizosphere.

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Abstract

The rhizosphere represents one of the most biologically active interfaces in terrestrial ecosystems, enriched with microorganisms capable of producing diverse secondary metabolites. In the present investigation, an antibiotic-producing bacterial isolate, Tomato Bacterial Strain (TBS), was isolated from the rhizosphere of tomato (*Solanum lycopersicum* L.) cultivated in Nagri, Ranchi, Jharkhand. Morphological, biochemical, enzymatic, and molecular analyses were performed to characterize the isolate. The colony exhibited large, blue-pigmented, rhizoid morphology with filamentous margins. Gram staining revealed Gram-positive rod-shaped cells. The isolate tested positive for catalase, oxidase, urease, starch hydrolysis, nitrate reduction, indole, methyl red, and Voges–Proskauer tests. Crude metabolite extracts showed strong antibacterial activity against *Escherichia coli* (19 mm), *Staphylococcus aureus* (14 mm), and *Bacillus subtilis* (12 mm), but no inhibition against *Pseudomonas aeruginosa*. Molecular identification using 16S rRNA gene sequencing confirmed its affiliation with the genus *Bacillus* (>99% similarity). The findings demonstrate that tomato rhizosphere soils of Jharkhand harbor metabolically versatile *Bacillus* species with promising antibacterial potential, supporting their role in antibiotic discovery and sustainable agricultural biocontrol strategies.

Keywords: Rhizosphere microbiology; *Bacillus*; Antibiotic-producing bacteria; Antimicrobial resistance; Tomato (*Solanum lycopersicum* L.); Soil microbiome; Secondary metabolites.

INTRODUCTION

Soil ecosystems represent one of the richest microbial reservoirs on Earth, containing billions of microorganisms per gram of soil [1,2]. Among soil microhabitats, the rhizosphere is particularly dynamic due to the influence of plant root exudates, which include sugars, amino acids, organic acids, and secondary metabolites that selectively enrich beneficial microbes [3,4]. The Solanaceae family, including tomato (*Solanum lycopersicum* L.), supports complex rhizospheric microbial interactions. These interactions often result in the enrichment of antibiotic-producing bacteria capable of suppressing phytopathogens [5]. Historically, soil microorganisms have contributed significantly to antibiotic discovery. Streptomycin, tetracycline, erythromycin, and vancomycin originated from soil bacteria [6,7,8,9]. However, the rapid emergence of antimicrobial resistance (AMR) threatens global health [10,11,12]. Multidrug-resistant pathogens such as MRSA and CRE demand novel antimicrobial agents.

Members of the genus *Bacillus* are well known for producing bioactive lipopeptides such as surfactin, iturin, and fengycin, which exhibit broad-spectrum antimicrobial properties [13,14]. Their ability to survive harsh soil conditions through endospore formation further enhances their ecological fitness [15].

Recent advances in natural product research emphasize microbial metabolites as a major source of novel drugs [16,17,18]. Given the agro-climatic diversity of Jharkhand and the underexplored rhizosphere microbiome, this study aimed to isolate, characterize, and molecularly identify a potent antibiotic-producing bacterium from tomato rhizospheric soil [19].

MATERIALS AND METHODS

Study Area and Soil Sampling

Soil samples were collected from tomato rhizosphere fields in Nagri, Ranchi district (10–15 cm depth) during the flowering stage. Samples were stored at 4°C.

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Isolation and Purification

Serial dilution (10^{-1}–10^{-9}) and spread plate technique on Nutrient Agar Medium were performed. Distinct colonies were purified using quadrant streaking. Rhizosphere soil samples were serially diluted up to 10^{-4}, and bacterial colonies were isolated. The isolate obtained from the 10^{-4} dilution was designated as TBS.

Morphological Characterization

TBS (10^{-4}) showed large colony size, blue pigmentation, rhizoid form, filamentous margins, flat elevation. Gram staining showed Gram-positive rod-shaped cells arranged singly.

Biochemical Characterization

Extracellular Tests

Extracellular biochemical tests were performed to determine enzyme activity of the isolates Catalase activity was tested by adding 3% hydrogen peroxide to bacterial culture and observing bubble formation. Oxidase test was conducted using tetramethyl-p-phenylenediamine reagent, where purple coloration indicated positivity. Gelatin hydrolysis was assessed by incubating isolates in nutrient gelatin and checking for liquefaction after refrigeration. Starch hydrolysis was determined by growing isolates on starch agar followed by iodine flooding to observe clear zones. Urease activity was tested on urea agar slants, where pink coloration indicated ammonia production. Litmus milk test was performed to observe acid production, proteolysis, or reduction based on color and texture changes after incubation.

Intracellular Tests

Intracellular biochemical tests were conducted to evaluate metabolic characteristics of the isolates. Nitrate reduction was determined using nitrate broth followed by addition of sulfanilic acid and α-naphthylamine reagents. Indole production was tested in tryptone broth using Kovac's reagent. Methyl Red and Voges–Proskauer tests were performed in MR-VP broth to detect mixed acid and acetoin production, respectively. Citrate utilization was examined on Simmons' citrate agar by observing blue color change. Triple Sugar Iron (TSI) test was carried out to determine sugar fermentation, gas production, and hydrogen sulfide formation based on color changes and black precipitate formation after incubation.

Extraction of Secondary Metabolites

Crude metabolites were extracted using ethyl acetate solvent extraction. Crude metabolites were extracted using ethyl acetate by first growing the bacterial culture in broth and then centrifuging it to separate the cell-free supernatant. An equal volume of ethyl acetate was added to the supernatant and mixed thoroughly to allow the metabolites to dissolve into the organic solvent. The mixture was then allowed to settle, forming two layers- aqueous and ethyl acetate. The upper ethyl acetate layer containing the metabolites was carefully collected and evaporated using a rotary evaporator or by air drying. The remaining residue obtained after evaporation was the crude metabolite extract, which was further used for antibacterial activity testing.

Antibacterial Assay

Disc diffusion method was performed following standard guidelines [20]. Disc diffusion method against:

- *Escherichia coli* (ACC-3099)
- *Staphylococcus aureus* (ACC-2408)
- *Pseudomonas aeruginosa* (ACC-3973)
- *Bacillus subtilis* (ACC-2511)

RESULTS

Morphological Identification

The isolate TBS (obtained from 10^{-4} dilution) showed significant results. The isolate TBS (10^{-4}) exhibited morphological characteristics showing large-sized colonies with blue pigmentation as shown in **Fig.1**. The colonies were rhizoidal in form, having filamentous margins and a flat elevation. Gram staining revealed that the isolate is Gram-negative, rod-shaped, and occurs singly as shown in **Fig.2**. Overall, TBS (10^{-4}) is characterized by distinct rhizoidal colony morphology and typical Gram-negative rod-shaped bacterial cells.

Figure 1. Pure culture

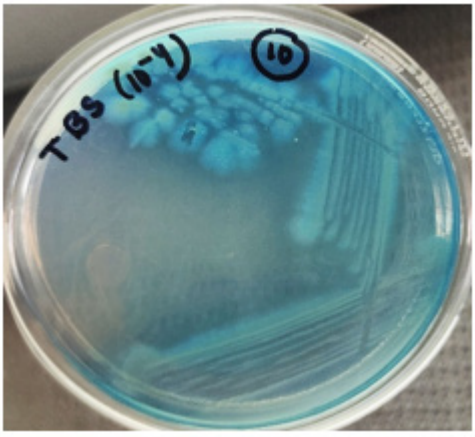
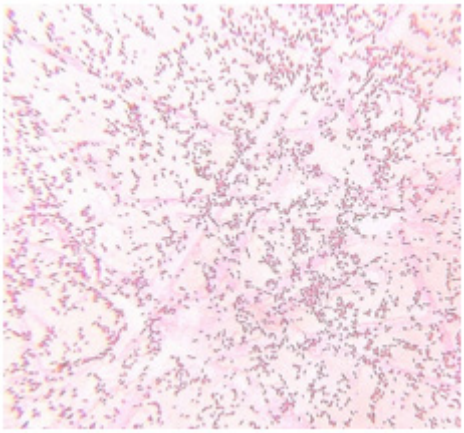


Figure 2. Gram staining showing grm negative rod-shaped.



Antibacterial Activity

Table 1. Zone of inhibition.

Pathogen	Zone (mm)
E. coli	19
S. aureus	14
B. subtilis	12
P. aeruginosa	0

Highest inhibition recorded against *E. coli* (255.26 mm² actual area). The antibacterial activity results indicate that the isolate exhibits strong inhibitory effect against *E. coli* (19 mm; highest activity), moderate activity against *S. aureus* (14 mm) and *B. subtilis* (12 mm), and no activity against *P. aeruginosa* (0 mm). Overall, the isolate is most effective against *E. coli* and shows selective antibacterial potential, suggesting possible production of bioactive compounds with a limited spectrum of activity.

Figure 3. Antibacterial activity of crude metabolite extract of *Bacillus* sp. TBS against selected bacterial pathogens measured by zone of inhibition.

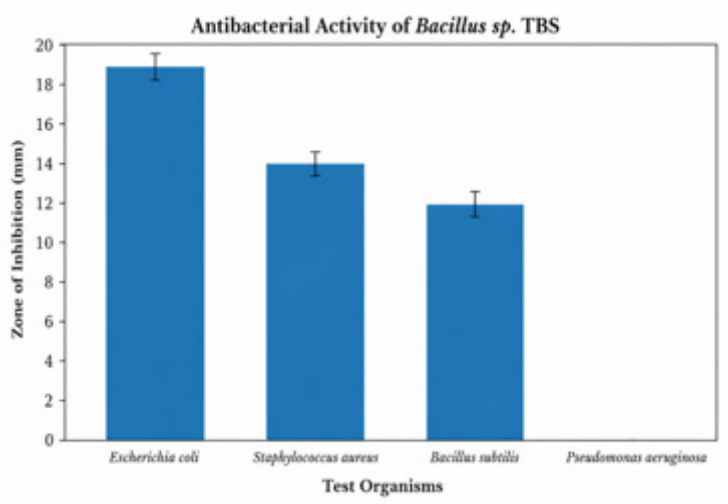
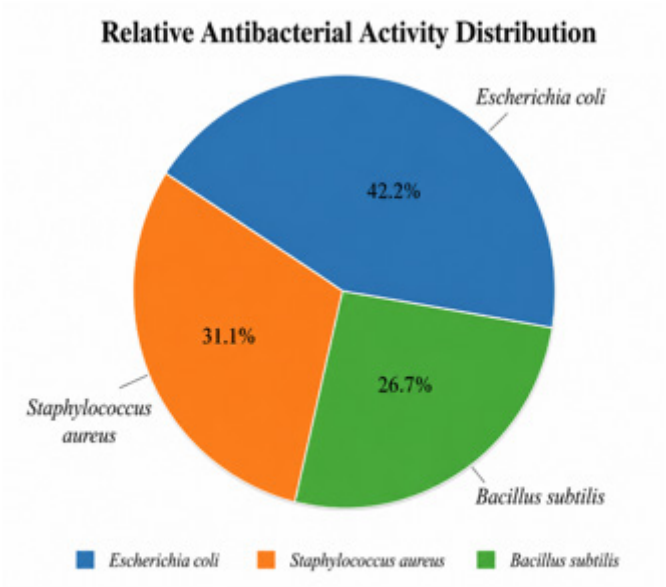


Figure 4. Relative antibacterial activity distribution of *Bacillus* sp. TBS crude metabolite extract against tested pathogens.



The **Figure 4** illustrates the relative antibacterial activity distribution of crude metabolite extracts produced by *Bacillus sp*. TBS against selected bacterial pathogens. Among the tested organisms, *Escherichia coli* exhibited the highest susceptibility,

accounting for 42.2% of the total antibacterial activity, indicating strong inhibitory potential of the isolate against Gram-negative bacteria. *Staphylococcus aureus* showed moderate susceptibility with 31.1% relative activity, while *Bacillus subtilis* exhibited comparatively lower inhibition with 26.7% activity.

The results demonstrate that the antibacterial metabolites produced by *Bacillus sp*. TBS possess broad-spectrum antimicrobial properties, with particularly strong effectiveness against *E. coli*. The absence of activity against *Pseudomonas aeruginosa* suggests selective antibacterial action, possibly due to the intrinsic resistance mechanisms of the pathogen such as efflux pumps and low membrane permeability. Overall, the findings support the potential application of rhizospheric Bacillus isolates as promising sources of bioactive antimicrobial compounds.

Biochemical Characterization

The biochemical characterization shows that the isolate is metabolically active, with positive results for catalase, oxidase, urease, starch hydrolysis, and litmus milk, indicating strong enzymatic activity and aerobic metabolism. It also shows positive intracellular reactions such as H_2S production, nitrate reduction, indole, and methyl red, suggesting active sulfur and nitrogen metabolism along with mixed acid fermentation. However, negative results for gelatin hydrolysis, carbohydrate fermentation (glucose, lactose, sucrose), and Voges-Proskauer indicate limited sugar utilization and absence of certain enzymes. Overall, the organism is versatile and relies more on enzymatic and alternative metabolic pathways.

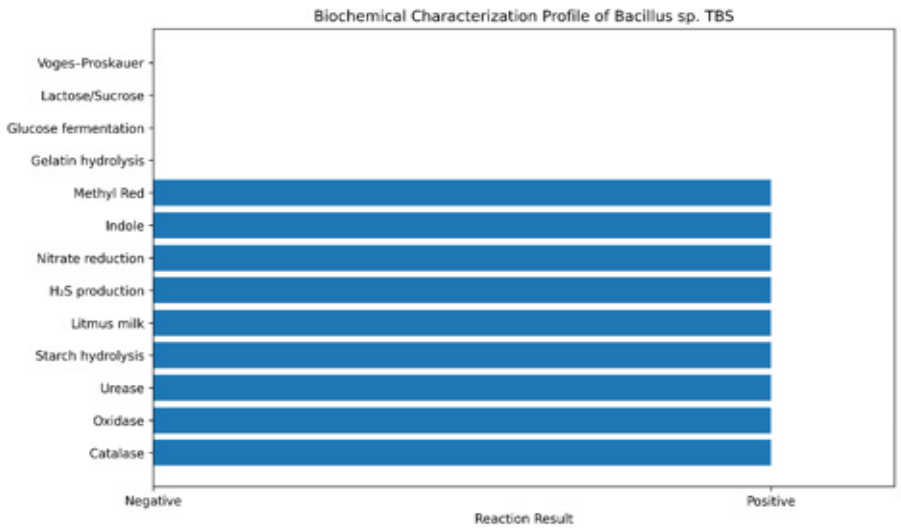
Table 2. Extracellular Tests.

Test	Result
Gelatin hydrolysis	Negative
Catalase	Positive
Oxidase	Positive
Urease	Positive
Starch hydrolysis	Positive
Litmus milk	Positive

Table 3. Intracellular Tests.

Test	Result
Glucose fermentation	Negative
Lactose/Sucrose	Negative
H_2S production	Positive
Nitrate reduction	Positive
Indole	Positive
Methyl Red	Positive
Voges–Proskauer	Negative

Figure 5. Biochemical characterization profile of *Bacillus sp*. TBS isolate showing positive and negative biochemical reactions.



The biochemical characterization profile of *Bacillus sp*. TBS demonstrated diverse metabolic and enzymatic activities. The isolate showed positive reactions for catalase, oxidase, urease, starch hydrolysis, litmus milk reaction, H_2S production, nitrate reduction, indole production, and methyl red test, indicating strong aerobic metabolism and active nitrogen and sulfur metabolic pathways. Positive starch hydrolysis and urease activities suggest the organism possesses extracellular enzymes capable of degrading complex substrates.

Negative reactions were observed for gelatin hydrolysis, glucose fermentation, lactose/sucrose fermentation, and Voges- Proskauer test, indicating limited carbohydrate fermentation ability and absence of acetoin production pathways. Overall, the biochemical profile confirms that *Bacillus sp*. TBS is metabolically versatile and possesses important physiological traits commonly associated with rhizospheric Bacillus species exhibiting antimicrobial potential.

DISCUSSION

The Gram-positive rod morphology, catalase positivity, and endospore-forming nature strongly align with genus Bacillus [15]. The positive oxidase and nitrate reduction tests indicate aerobic respiratory metabolism. Such biochemical versatility is characteristic of soil-dwelling *Bacillus* species [19]. The strong inhibition against Gram-negative *E. coli* suggests production of membrane-disrupting metabolites such as lipopeptides and other secondary metabolites [13, 9]. These compounds are widely reported for their antimicrobial efficacy and industrial importance [17, 14]. Lack of activity against *Pseudomonas aeruginosa* is likely due to intrinsic resistance mechanisms such as efflux pumps and low membrane permeability [21, 11].

The rhizosphere-driven enrichment of bioactive strains supports plant–microbe co-evolution and functional microbiome interactions [5, 4]. Such microbial diversity represents an untapped reservoir for novel antibiotic discovery [16, 18].

CONCLUSION

Bacillus sp. TBS (10^{-4}) from tomato rhizosphere exhibits strong antibacterial potential and diverse enzymatic activity. This isolate represents a promising candidate for novel antibiotic discovery and sustainable agricultural biocontrol applications.

Conflict of interest

The author declares no potential conflict of interest with respect to the research, authorship, and publication of this article.

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