Molecular Identification and characterization of *Bacillus cereus* Strain BT-1 isolated from Buffalo Dung - East Godavari District, Andhrapradesh.

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ABSTRACT

Buffalo dung is a rich source of diverse microorganisms with potential applications in agriculture, biotechnology, and environmental management. The present study was undertaken to isolate molecular characterization of a bacterial strain from the buffalo dung collected from Devarapalli, East Godavari District, Andhra Pradesh, India. The sample was processed using the serial dilution technique, followed by culturing on Nutrient Agar medium. A distinct bacterial colony was purified through repeated streak plating to obtain a pure culture and Bacterial culture. Genomic DNA do the Extraction of **16s ribosomal RNA (rRNA) gene** was amplified using universal bacterial primers and sequenced. The obtained sequence results was analyzed using the NCBI BLAST program, The NCBI Results showed that the isolate belongs to ***Bacillus cereus* strain BT-1**. The nucleotide sequence was submitted to the NCBI GenBank database and assigned the accession number **PV202403.1**. Members of the genus *Bacillus* are well known for their ability to produce industrially important enzymes, antimicrobial compounds, plant growth-promoting metabolites, and spores that enhance environmental adaptability. The successful isolation and molecular characterization of *Bacillus cereus* strain BT-1 demonstrate the microbial diversity present in buffalo dung and highlight its potential as a valuable source of beneficial bacteria.

Keywords: *Bacillus cereus*, Buffalo dung, 16s rRNA gene sequencing, bacterial isolation, molecular identification, GenBank accession PV202403.1, microbial diversity, biotechnology.

Introduction

Buffalo dung is a rich and dynamic microbial ecosystem that harbors a wide variety of bacteria, fungi, actinomycetes, and other microorganisms involved in the decomposition of organic matter and nutrient recycling. Owing to its high content of partially digested plant materials, cellulose, hemicellulose, proteins, minerals, and moisture, buffalo dung provides an ideal environment for the proliferation of diverse microbial populations. These microorganisms play essential roles in maintaining soil fertility, improving nutrient availability, promoting plant growth, and supporting ecological sustainability. Consequently, livestock dung has become an important source for the isolation of microorganisms with potential applications in agriculture, environmental management, pharmaceuticals, and industrial biotechnology (1).

Among the microorganisms commonly isolated from environmental samples, members of the genus *Bacillus* are of particular scientific and industrial importance. The genus *Bacillus* comprises Gram-positive, rod-shaped, aerobic or facultatively anaerobic, endospore-forming bacteria belonging to the family Bacillaceae (2). The ability to form highly resistant endospores enables these bacteria to survive under adverse environmental conditions such as desiccation, nutrient limitation, high temperature, and chemical stress (3). Because of their remarkable physiological adaptability, *Bacillus* species are widely distributed in soil, water, animal waste, plant rhizospheres, and various natural habitats.

Bacillus cereus is one of the most extensively studied species within the genus due to its ecological versatility and metabolic diversity. Although certain strains are recognized as foodborne pathogens capable of producing enterotoxins responsible for emetic and diarrheal syndromes (4), numerous environmental isolates exhibit beneficial properties with significant industrial potential (5). These include the production of extracellular enzymes such as amylases, proteases, cellulases, xylanases, lipases, and pectinases, as well as antimicrobial compounds, biosurfactants, and bioactive metabolites (6). Several *Bacillus* strains have also been investigated for applications in bioremediation, composting, biodegradation of agricultural wastes, biofertilizer production, and plant growth promotion.

Accurate identification of bacterial isolates is fundamental for understanding their taxonomy, ecological significance, and potential applications.

Conventional identification methods based on colony morphology and biochemical characteristics often fail to distinguish closely related bacterial species due to phenotypic variability(7). Molecular identification based on sequencing of the 16S ribosomal RNA (16S rRNA) gene has therefore become the most reliable and widely accepted method for bacterial taxonomy. The highly conserved nature of the 16S rRNA gene, together with its hypervariable regions, enables accurate species-level identification and phylogenetic analysis

Methodology

Sample Collection Collection Sites

- Devarapalli
- East Godavari district

The first step of our study was sample collection. Fresh buffalo dung samples were collected from two

different locations, **Devarapalli**. Fresh samples were collected in sterile containers and transported immediately to the laboratory for microbial analysis.

Collecting samples from different locations increases the possibility of isolating diverse bacterial species.

Serial Dilution and Culturing

The collected dung samples contained millions of microorganisms. Therefore, serial dilution was performed to reduce the bacterial concentration.

Process

- One gram of dung was mixed with sterile distilled water.
- The suspension was diluted step by step from 10^{-1} to 10^{-9}.
- Suitable dilutions were spread onto nutrient agar plates.
- The plates were incubated at **37°C for 16–24 hours**.

Serial dilution helps to obtain isolated colonies are easy to study.

Streaking

After incubation, individual colonies were selected and streaked on fresh nutrient agar plates using the streak plate technique.

The objectives of streaking are:

- To obtain isolated pure single colony,
- To produce pure bacterial cultures,

- Do the sub culturing of bacterial culture.

Colony Formation

After the subculture do for bacterial plating (or) Smearing of the culture in a plates were incubated at **37°C for 16–24 hours**.

After incubation, bacterial colonies appeared on the agar plates. The colonies were examined based on:

- Shape
- Size
- Colour
- Margin
- Elevation
- Surface texture
- Pigmentation

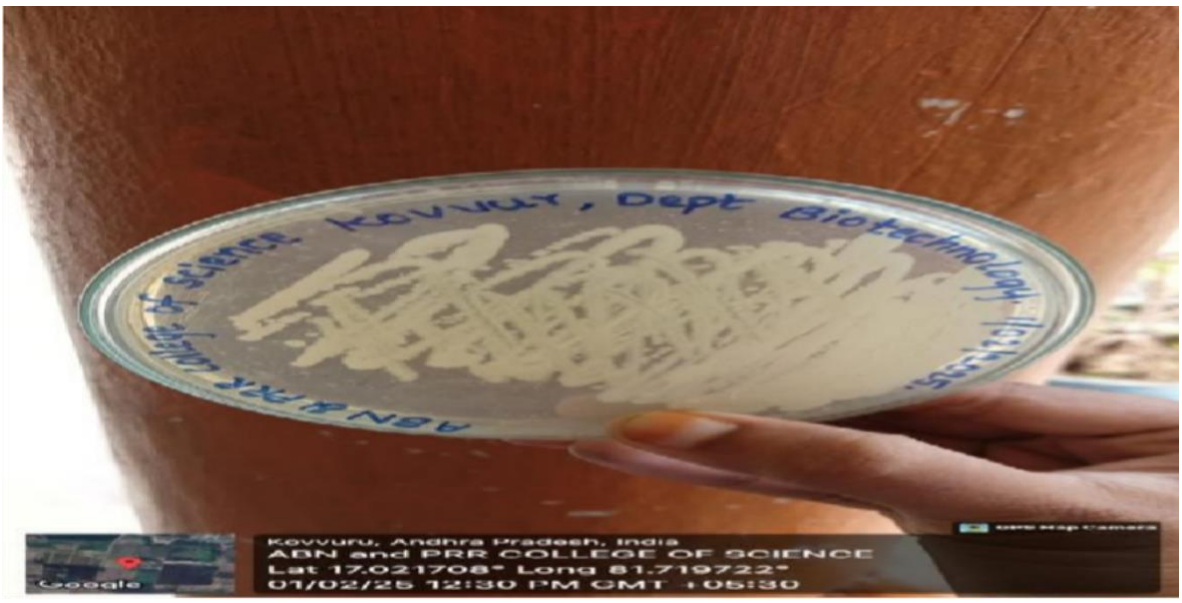
Each colony originates from a single colony-forming unit (CFU) process was performed. The variation in colony morphology indicates the presence of different bacterial species. **Sub-culturing**

Well-isolated colonies were transferred onto fresh nutrient agar slants by sub-culturing in the bacteria.

The purpose of sub-culturing is:

- To maintain pure bacterial cultures
- To preserve bacterial isolates
- To perform further molecular identification

Pure cultures can also be stored for future research



Result

The study successfully isolated several bacterial colonies from buffalo dung samples collected from both locations.The isolates showed different colony characteristics, indicating microbial diversity.Pure cultures were successfully obtained through repeated streaking and sub-culturing.

These isolates can be further analyzed using the following techniques

- Gram staining, Molecular identification using by 16S rRNA sequencing method

By the gram staining the *Bacillus cereus* Strain BT-1 is identified as Gram Positive Bacteria under the microscopic observation.

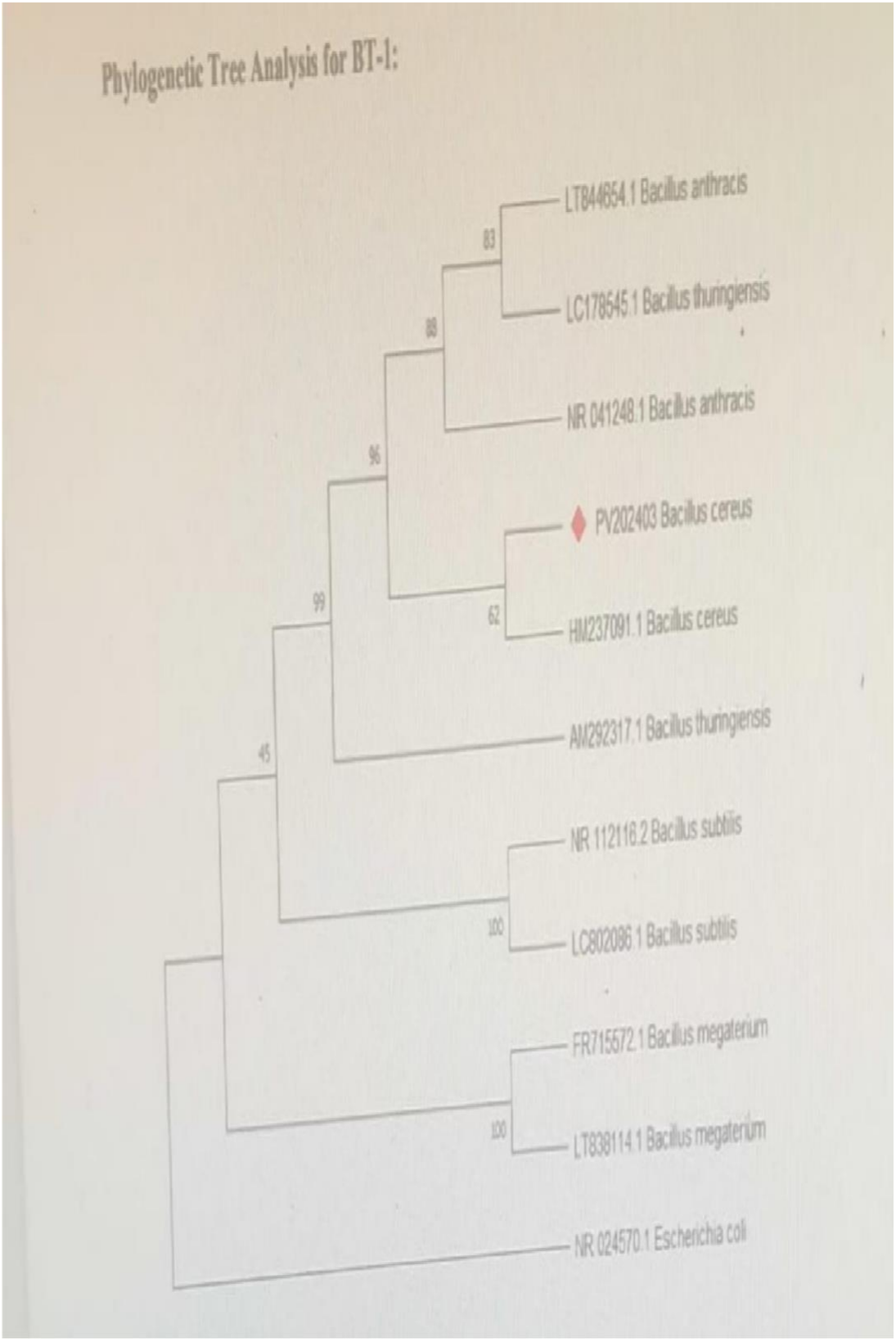
The overall study demonstrated that buffalo dung is an excellent natural source of beneficial microorganisms.The experimental studies reveal that the buffalo dung is one of the natural source of beneficial microorganisms. Used for different applications.

Future studies are required to identify the bacterial isolates and evaluate their potential applications in agriculture, and environmental management.

Based on the Gen Bank records uploaded, the organisms presented are:

BT-1

- **Organism:** Bacillus cereus
- **Identification:** 16S rRNA gene sequencing
- **GenBank Accession:** PV202403.1
- **Isolation Source:** Buffalo dung
- **Collection Site:** Devarapalli, East Godavari District, Andhra Pradesh, India.



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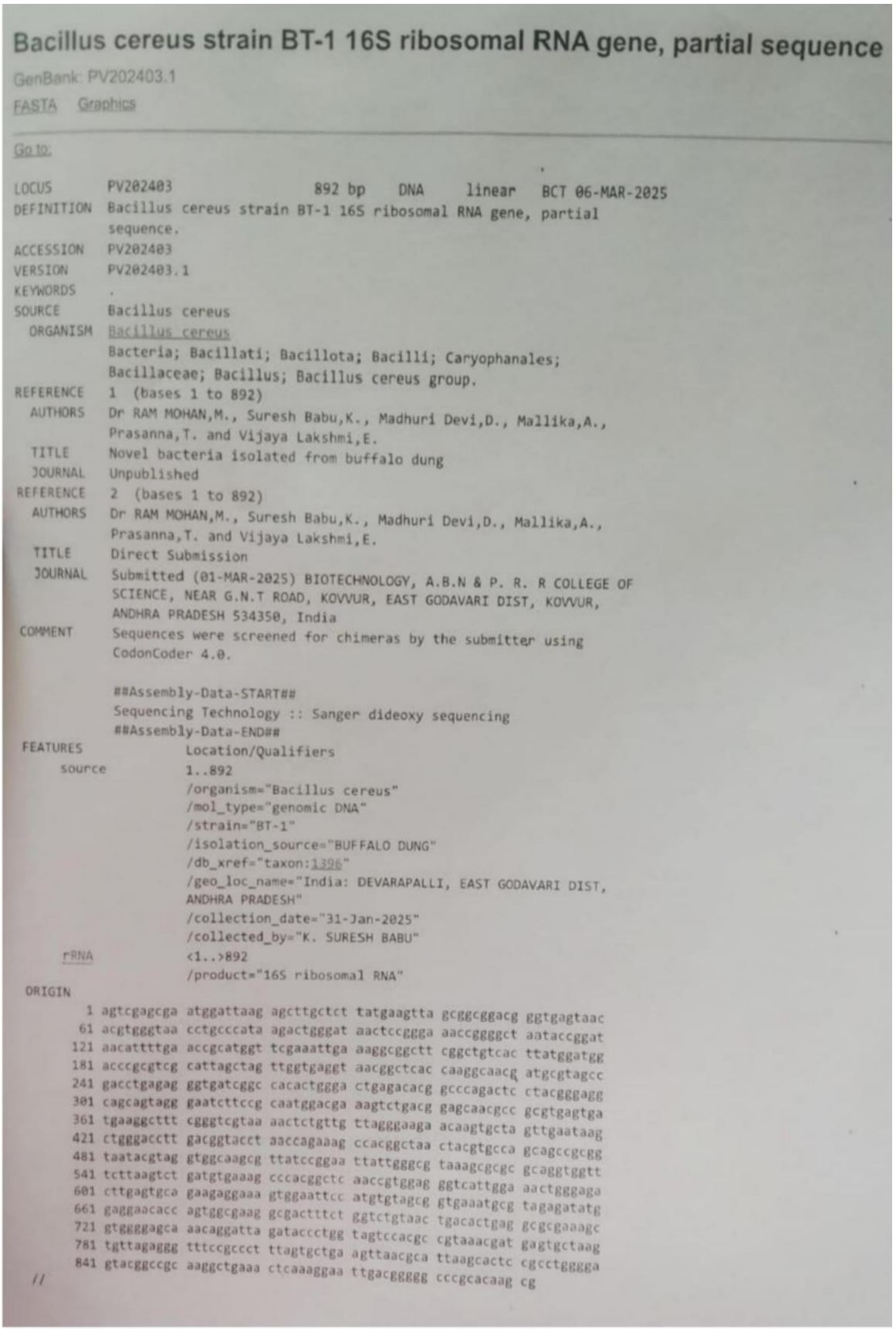

Bacillus cereus strain BT-1 16S ribosomal RNA gene, partial sequence

GenBank: PV202403.1

FASTA Graphics

Go to:

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DEFINITION  Bacillus cereus strain BT-1 16S ribosomal RNA gene, partial
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ACCESSION   PV202403
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REFERENCE   1  (bases 1 to 892)
  AUTHORS   Dr RAM MOHAN,M., Suresh Babu,K., Madhuri Devi,D., Mallika,A.,
            Prasanna,T. and Vijaya Lakshmi,E.
  TITLE     Novel bacteria isolated from buffalo dung
  JOURNAL   Unpublished
REFERENCE   2  (bases 1 to 892)
  AUTHORS   Dr RAM MOHAN,M., Suresh Babu,K., Madhuri Devi,D., Mallika,A.,
            Prasanna,T. and Vijaya Lakshmi,E.
  TITLE     Direct Submission
  JOURNAL   Submitted (01-MAR-2025) BIOTECHNOLOGY, A.B.N & P. R. R COLLEGE OF
            SCIENCE, NEAR G.N.T ROAD, KOVVUR, EAST GODAVARI DIST, KOVVUR,
            ANDHRA PRADESH 534350, India
COMMENT     Sequences were screened for chimeras by the submitter using
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Discussion

The present study successfully isolated and molecularly identified *Bacillus cereus* strain BT-1 from buffalo dung using conventional microbiological techniques followed by 16S rRNA gene sequencing with Phylo genetic Tree analysis(8). Buffalo dung represents a nutrient-rich ecological niche containing a diverse consortium of microorganisms originating from the gastrointestinal tract of herbivorous animals and the surrounding environment (9). The successful isolation of strain BT-1 further confirms that buffalo dung serves as an important natural reservoir of beneficial bacteria with ecological and industrial significance(9). This study contributes to the understanding of microbial diversity associated with buffalo dung and provides a valuable bacterial isolate for future investigations aimed at evaluating its physiological, biochemical, and biotechnological potential.

Serial dilution and nutrient agar cultivation facilitated the development of well-isolated bacterial colonies, while repeated streak plating ensured the establishment of a pure culture suitable for molecular characterization (10). The variation observed in colony morphology during isolation reflects the high microbial diversity present in buffalo dung, which has been reported by several investigators as a promising source of beneficial microorganisms (11).

Molecular identification based on 16S rRNA gene sequencing confirmed the isolate as *Bacillus cereus* strain BT-1. The nucleotide sequence exhibited a high degree of similarity with previously reported *B. cereus* sequences available in the NCBI GenBank database and was assigned the accession number **PV202403.1**. The use of 16S rRNA sequencing significantly improves the accuracy of bacterial identification compared with conventional biochemical methods and has become the standard procedure for bacterial taxonomy and phylogenetic studies.

The genus *Bacillus* is well known for producing a wide range of extracellular hydrolytic enzymes including amylases, proteases, cellulases, lipases, xylanases, and pectinases that have extensive applications in food processing (12), textile manufacturing, detergent formulation, paper production, biofuel generation(13), and waste management. Environmental isolates of *Bacillus cereus* (14) have also been reported to produce antimicrobial compounds capable of inhibiting several bacterial and fungal pathogens (15).

In addition, many *Bacillus* species contribute to nutrient cycling, organic matter decomposition, phosphate solubilisation, and plant growth promotion, making them attractive candidates for sustainable agricultural practices (16).

Although *Bacillus cereus* is commonly associated with food poisoning through the production of emetic and diarrheal toxins (17), not all environmental isolates possess significant pathogenic potential. Therefore, comprehensive physiological, biochemical, genomic, and virulence analyses are necessary before considering industrial or agricultural applications. Evaluation of toxin genes(18), antimicrobial susceptibility, enzyme production(19), biosafety assessment(20), and whole-genome sequencing would provide a more comprehensive understanding of the functional characteristics of strain BT-1.

The successful deposition of the nucleotide sequence in the NCBI GenBank database provides an authenticated molecular record of the isolate and contributes valuable genetic information and accessible in microbial databases. Future studies should investigate the isolate's enzyme production profile (21), antimicrobial metabolite synthesis, biodegradation capability, plant growth-promoting characteristics, and genomic diversity to fully explore its potential in biotechnology and environmental management.

Conclusion

The present investigation successfully isolated and has done Molecular characterization identified *Bacillus cereus* strain BT-1 from buffalo dung collected from Devarapalli, East Godavari District, Andhra Pradesh, India. Standard microbiological methods, including serial dilution, nutrient agar cultivation, and repeated streak plating, enabled the isolation of a pure bacterial culture, while molecular characterization through 16S rRNA gene sequencing accurately confirmed taxonomic identity by the phylogenetic tree analysis(22). The nucleotide sequence was successfully deposited in the NCBI GenBank database under Accession Number **PV202403.1**, providing a permanent genetic record of the isolate(23). This findings demonstrate that buffalo dung is an important natural reservoir of diverse bacterial species with potential applications in agriculture, environmental biotechnology, and industrial microbiology. Furthermore it will be used for enzyme production(24), antimicrobial activity, biodegradation potential, plant growth-promoting properties, for the practical significance of *Bacillus cereus* strain BT-1. In the Future aspect, this organism studies on the physiological, biochemical, and functional properties of this isolate may reveal its suitability for applications in agriculture, biodegradation, enzyme production, and will be used for sustainable applications.

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