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Isolation and functional characterization of plant-growth-promoting bacteria from indigenous Hariana cow dung for sustainable agricultural applications

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Abstract
This study investigated the microbial potential of indigenous Hariana cow dung for sustainable agricultural applications. Microbial isolates from Hariana dung demonstrated multifunctional traits, including zinc, phosphate, and potassium solubilization, along with nitrogen fixation. Noteworthy strains such as *Brachybacterium paraconglomeratum*, *Shigella dysenteriae*, *Bacillus safensis* and *Escherichia marmotae* were identified, highlighting the diversity and functional richness of the microbial community. These isolates (except *S. dysenteriae*: pathogenic) may contribute to nutrient mobilization and organic matter decomposition, thereby enhancing soil fertility and promoting plant growth. The presence of multi-activity strains offered a promising alternative to chemical fertilizers by integrating nutrient solubilization with biological nitrogen enrichment. Observations also revealed significant variation in microbial traits, underscoring the value of indigenous cattle as reservoirs of plant-growth-promoting bacteria. This work lays the foundation for harnessing indigenous microbiota derived from native cow dung to develop biofertilizers to improve soil health for better productivity.

Keywords: Indigenous cows, Hariana cow dung, nutrient solubilization activities, plant-growth-promoting bacteria, antagonistic activity

1. Introduction
India has a rich agricultural heritage that includes the traditional use of cow dung and urine as natural fertilizers and insecticides. These practices, deeply rooted in indigenous farming systems, have been validated by recent scientific studies for their effectiveness in promoting sustainable agriculture. Cow dung is not merely organic waste—it is a biologically active substance containing a wealth of organic matter, essential nutrients such as nitrogen, phosphorus, and potassium, and a diverse population of beneficial microorganisms. These components collectively contribute to improved soil structure, enhanced fertility, and robust plant growth.

India's cattle population stands at approximately 193.46 million, which accounts for 14.5% of the global cow population and 36.04% of the country's total livestock (DAHD, 2022) [1]. Of these, 142.11 million cows—representing 73.45%—are indigenous breeds, which have evolved over generations to adapt to local climatic and nutritional conditions. India boasts 53 recognized indigenous cow breeds, making it the most genetically diverse cattle nation in the world (ICAR, 2024) [2].

These native breeds, such as the Hariana, are known for their resilience to heat, disease resistance, and ability to thrive under nutritional stress. The Hariana breed is particularly valued for its dual-purpose utility: it provides milk for human consumption and bullocks for draught power. However, due to increased agricultural mechanization and the breed's relatively low milk yield, many of these cows are underutilized. Farmers often sell them to traders, abandon them in nearby forests, or allow them to roam freely along roadsides [3]. To address this issue, there is a growing need to explore alternative uses of indigenous cattle that offer economic and ecological benefits. One promising avenue is the utilization of cow dung from native breeds for microbial

enrichment and soil health improvement. The manure of indigenous cows, particularly Hariana, has been found to contain enhanced microflora due to their adaptive traits, making it a valuable resource for organic farming [4].

Cow dung is rich in Plant Growth-Promoting Bacteria (PGPB), which inhabit the rhizosphere—the soil region surrounding plant roots—and play a crucial role in stimulating plant development. These bacteria include *Rhizobium* sp., *Azotobacter* sp., *Pseudomonas* sp., and *Bacillus* sp., each contributing to vital soil functions such as nitrogen fixation, solubilization of phosphorus, potassium, and zinc, and production of siderophores that facilitate nutrient uptake. Additionally, these microbes synthesize plant growth hormones like auxins, cytokinins, and gibberellins, which further enhance root and shoot development [5].

The application of cow dung as a biofertilizer not only improves crop yield but also contributes to long-term soil health by increasing organic matter content and promoting nutrient cycling [6]. Unlike synthetic fertilizers, which can degrade soil quality over time, cow dung offers a sustainable alternative that supports ecological balance and agricultural productivity [7].

Moreover, the lower digestive tract of cows harbors a diverse microbial ecosystem with probiotic activity. Microorganisms such as *Lactobacillus plantarum*, *Lactobacillus casei acidophilus*, *Bacillus subtilis*, *Enterococcus diactylactis*, *Bifidobacterium*, and *Saccharomyces cerevisiae* contribute to the health of the cow and enhance the quality of its excreta (Tomar *et al*., 2020) [8]. Bacterial population from excreta has also demonstrated strong antibacterial activity, particularly against Gram-positive bacteria, making it a valuable input for integrated pest management [9].

Vermicomposting is another sustainable technique that can be employed to enhance the microbial richness of cow dung. This process involves the use of earthworms to decompose organic waste, resulting in compost that is rich in plant growth stimulants and beneficial microbes. When cow dung from Hariana cattle is used in vermicomposting, it can be further enriched by inoculating it with isolated PGPB strains from the same breed's dung. These microbial inoculants can be applied individually or as consortia to improve plant growth and reduce pest infestation [10].

To fully harness the potential of native cow microbiota, a structured research initiative is essential. This initiative should begin with the isolation, identification, and characterization of microorganisms from fresh Hariana cow dung. Simultaneous composting of the same cow's excreta will allow for assessment of microbial durability and enrichment. The study should focus on evaluating nutrient-solubilizing capabilities—specifically zinc, potassium, and phosphate—along with nitrogen fixation activity. Biochemical and molecular characterization of these microbes will help determine their antagonistic properties against plant pathogens and their overall efficacy in promoting plant health [11].

By leveraging the microbial potential of indigenous cattle, particularly the Hariana breed, this research supports the development of sustainable agricultural practices and offers new opportunities for rural livelihoods. It also provides a compelling alternative to the abandonment or sale of native cows, encouraging farmers to recognize and utilize their broader ecological and economic value.

2. Materials and Methods

2.1 Sample Collection

A sterile polybag was used to collect a fresh sample of Hariana cow dung from the middle of the ejected excrement. In order to prevent sample saturation, collected samples were processed for analysis within 24 hours, normally.

2.2. Media Preparation & Sample Inoculation

Plate Count Agar (PCA), Violet Red Bile Agar (VRBA), Chloramphenicol Yeast Glucose Agar (CYGA), and Minimum Recovery Diluent (MRD) were prepared as microbiological media for the initial processing steps. All media, except VRBA, were sterilized by autoclaving at 121 °C for 15 minutes under a pressure of 15 PSI. VRBA was prepared by boiling and directly pouring into plates without autoclaving. For sample preparation, 90 ml of MRD was mixed with 10 grams of cow dung. This mixture served as the base for microbial isolation. The isolation procedure was carried out using the serial dilution method to reduce microbial concentration and facilitate accurate enumeration. After completing the serial dilution, one milliliter from each dilution was transferred in duplicate to sterile Petri plates for microbial quantification. These plates were used to estimate the Total Bacterial Count (TBC), Total Yeast and Mold Count (Y&MC), and Total Coliform Count (TCC). Sterilized PCA and CYGA, along with freshly boiled VRBA, were poured into the sample-containing plates maintained at a temperature range of 45-50 °C using the pour plate technique. The contents of each plate were gently mixed in both clockwise and counterclockwise directions to ensure uniform distribution and prevent spillage. Once the media solidified, the PCA and CYGA plates were incubated at their respective optimal temperatures—30 °C for PCA and 25 °C for CYGA. PCA plates were incubated for three days to allow bacterial growth, while CYGA plates were incubated for five days to support yeast and mold development. After solidification of the VRBA plates, 4 ml of additional VRBA was layered onto each plate. These were thoroughly mixed, allowed to solidify again, and then incubated at 37 °C for 24 hours to facilitate coliform detection.

2.3. Isolation and identification

After 24 hours of incubation at 37°C, the VRBA plates were examined to estimate the Total Coliform Count (TCC). The observed results were carefully recorded for further analysis. Following three days of incubation, bacterial colonies that exhibited visible growth on PCA and CYGA plates—prepared from dung samples—were identified based on distinct morphological characteristics. These colonies were selected for further characterization to assess microbial diversity and functional traits. After five days of incubation, the CYGA plates were evaluated for proper fungal development. The growth of yeast and mold was assessed to ensure colony integrity and consistency. Fungal colonies were selected based on morphological variations, such as changes in texture, pigmentation, and colony structure, which became evident after the five-day incubation period. In parallel, quantitative estimations of Total Bacterial Count (TBC) and Total Yeast and Mold Count (Y&MC) were conducted using the respective PCA and CYGA plates. These analyses were performed for dung samples to determine microbial load and diversity across sample types. The results provided a comprehensive overview of the microbial populations present in the samples and their potential relevance for agricultural applications.

2.4. Strain Characterization

A total of 10 microbial isolates were selected for detailed strain characterization. The primary objective of this research is to establish agriculture-friendly microbial consortia, with a

particular emphasis on morphological traits and inter-strain variations. To achieve this, the selected isolates were initially screened for their qualitative ability to solubilize key nutrients such as zinc, potassium, and phosphate, as well as their capacity for nitrogen fixation. Isolates that demonstrated positive results in these preliminary functional assays were shortlisted for further analysis. These promising strains underwent biochemical characterization to determine their metabolic profiles and enzymatic activities relevant to plant growth promotion. In addition, their antagonistic potential was evaluated to assess their ability to inhibit phytopathogenic organisms, which is crucial for integrated pest management. Finally, molecular characterization techniques were employed to identify the strains at the genetic level, ensuring precise taxonomic classification and understanding of their functional genes. This multi-tiered approach enables the selection of robust microbial candidates for the development of effective and sustainable biofertilizer consortia tailored to agricultural applications.

2.4.1 Zinc Solubilizing Activity

Zinc solubilizing activity was assessed using Zinc Solubilizing Agar (Himedia-M2068), which contains dextrose (glucose), ammonium sulphate, potassium chloride, dipotassium hydrogen phosphate, magnesium sulphate heptahydrate, zinc oxide, and agar. The medium was adjusted to a pH of 7.2 to support optimal microbial growth and solubilization conditions. Prior to use, the agar was sterilized by autoclaving at 121°C for 15 minutes under a pressure of 15 psi to ensure complete elimination of contaminants. To evaluate zinc solubilization, selected bacterial isolates were directly streaked onto the solidified and contamination-verified agar plates. These inoculated plates were incubated at 30 °C for a period of two days. For isolates exhibiting slower growth rates, the incubation was extended to four to five days to allow sufficient colony development. Zinc-solubilizing strains were identified by the presence of a clear halo zone surrounding the bacterial colony. This zone indicates the solubilization of insoluble zinc compounds into bioavailable forms. The extent of solubilizing activity was quantified by measuring the diameter of the halo zone around each colony, providing a visual and measurable indicator of the strain's zinc-mobilizing potential [12, 13].

2.4.2 Potassium Solubilizing Activity

Potassium solubilizing activity was evaluated using Aleksandrow agar (Himedia-M1996), a specialized medium formulated to detect microbial potassium mobilization. The medium composition included dextrose (glucose), magnesium sulphate, ferric chloride, calcium carbonate, calcium phosphate, and potassium alumino-silicate, with agar as the solidifying agent. The pH of the medium was adjusted to 7.2 to support optimal microbial growth and solubilization conditions. To ensure sterility, the medium was autoclaved at 121°C for 15 minutes under a pressure of 15 PSI. Following sterilization and solidification, the medium plates were carefully inspected to confirm the absence of contamination. Selected bacterial isolates were then directly streaked onto the surface of the prepared plates. These inoculated plates were incubated at 28°C for a period of seven days to allow sufficient time for colony development and potassium solubilization. After the incubation period, the plates were examined for the presence of halo zones surrounding the bacterial colonies. These clear zones indicate the solubilization of insoluble potassium compounds into bioavailable forms. The diameter of the halo zone was measured to quantify the potassium solubilizing activity of each isolate, providing a visual and functional assessment of their nutrient-mobilizing potential [14].

2.4.3 Phosphate Solubilizing Activity

Phosphate solubilizing activity was assessed using Pikovskaya's agar (Himedia-M520), a specialized medium designed to detect microbial phosphate mobilization. The medium composition included yeast extract, dextrose (glucose), magnesium sulfate, potassium chloride, calcium phosphate, ammonium sulphate, manganese sulphate, and ferrous sulphate. The pH of the medium was carefully adjusted to 7.2 to support optimal microbial growth and solubilization conditions. To ensure sterility and eliminate any microbial contaminants, the agar was autoclaved at 121°C for 15 minutes under a pressure of 15 PSI. Once the medium had solidified and passed contamination checks, selected bacterial isolates were directly streaked onto the surface of the plates. These inoculated plates were incubated at 37°C for a period of five days to allow sufficient time for colony development and phosphate solubilization. After the incubation period, the plates were examined for the presence of clear halo zones surrounding the bacterial colonies. These zones indicated the solubilization of insoluble calcium phosphate into bioavailable forms. The diameter of the halo zone was measured to quantify the phosphate solubilizing activity of each isolate, providing a visual and functional assessment of their nutrient-mobilizing potential (Yu *et al.*, 2022) [15].

2.4.4 Nitrogen fixation Activity

Nitrogen fixation activity was assessed using Norris Glucose Nitrogen-Free Medium (Himedia-M712), which is specifically formulated to detect diazotrophic bacterial strains capable of fixing atmospheric nitrogen. The medium composition included ferrous sulphate, dipotassium hydrogen phosphate, magnesium sulphate, calcium carbonate, sodium chloride, sodium molybdate, and dextrose (glucose), with agar serving as the solidifying agent. The pH of the medium was adjusted to 7.0 to support optimal microbial growth under nitrogen-deficient conditions. To ensure sterility, the medium was autoclaved at 121°C for 15 minutes at a pressure of 15 PSI. Once the medium had solidified and was confirmed to be free of contamination, selected bacterial isolates were directly streaked onto the surface of the plates. These inoculated plates were incubated at 28°C for a period of seven days to allow sufficient time for colony development and nitrogen fixation activity to manifest. After the incubation period, the plates were examined for the presence of halo zones surrounding the bacterial colonies. These clear zones indicated nitrogen fixation activity, as the bacteria converted atmospheric nitrogen into bioavailable forms in the absence of external nitrogen sources. The diameter of the halo zone was measured to quantify the nitrogen-fixing potential of each isolate, providing a visual and functional assessment of their diazotrophic capabilities [16]. A total of fifteen distinct biochemical tests were conducted to evaluate their physiological and metabolic properties. Each isolate was inoculated into appropriate media and incubated overnight at 37°C to ensure sufficient bacterial growth. To determine the Gram reaction, the overnight-grown cultures were subjected to Gram staining and observed under a compound microscope at 100X magnification. This procedure enabled the differentiation of Gram-positive and Gram-negative bacteria based on their cell wall structure and staining behavior.

2.5 Biochemical Characterization

Following Gram staining, the isolates were tested for oxidase

and catalase activity. The oxidase test was used to identify bacteria capable of producing cytochrome oxidase, an enzyme involved in the electron transport chain that facilitates the reduction of oxygen to water. For this test, filter paper was soaked in a 1% solution of tetra-methyl-p-phenylenediamine dihydrochloride, a synthetic electron donor, and then dried. Bacterial colonies were spread onto the paper, and a color change to dark purple within ten seconds indicated a positive oxidase reaction [17].

Catalase activity was assessed using 3% hydrogen peroxide (H_2O_2). The presence of catalase enzyme was confirmed by the release of oxygen bubbles upon contact with H_2O_2, indicating the breakdown of hydrogen peroxide into water and oxygen. This test helped distinguish catalase-producing bacteria from non-producers.

Additional biochemical tests were performed to further characterize the isolates. These included the Indole test using peptone broth to detect tryptophanase activity, and the Methyl Red and Voges-Proskauer tests using MR-VP medium to evaluate mixed acid fermentation and acetoin production. Lactose fermentation was assessed using lactose broth, while gas production was observed in nutrient broth through bubble formation.

The Urease test was conducted using urease medium to detect urea hydrolysis. Eosin Methylene Blue (EMB) agar was used to differentiate lactose fermenters and identify Gram-negative enteric bacteria. Mannitol Salt Agar (MSA) was employed to assess mannitol fermentation and salt tolerance. Citrate utilization was tested by streaking the isolates on Simmons citrate agar to determine their ability to use citrate as a sole carbon source.

Triple Sugar Iron (TSI) agar was used to evaluate the fermentation of dextrose, lactose, and sucrose, as well as the production of hydrogen sulfide (H_2S). All cultures were incubated at 37°C for 24 hours to allow for the expression of biochemical traits. The results from these tests provided a comprehensive profile of each isolate's metabolic capabilities and potential applications in agriculture.

2.6 Antagonistic Properties

To evaluate the antagonistic effect of selected bacterial isolates, the fungus *Aspergillus brasiliensis* was used as the test organism. All bacterial strains were assessed for their ability to inhibit fungal growth. A well-established fungal slant of *A. brasiliensis* was suspended in a polysorbate solution to prepare the fungal inoculum. From this suspension, 0.1 ml was absorbed onto sterile cotton discs to serve as the fungal source. Mueller-Hinton Agar (MHA) and Sabouraud Dextrose Agar (SDA) were prepared and combined in equal proportions to create a dual-nutrient medium suitable for both bacterial and fungal growth. This composite medium was used to facilitate the antagonistic interaction between the bacterial isolates and the fungus. Each labeled Petri plate contained an overnight-grown bacterial culture streaked across the surface. The fungal-loaded cotton disc was then placed at the center of each plate to initiate the antagonism assay. The plates were incubated at 30 °C for five days to allow for sufficient interaction and observable growth patterns. After incubation, the plates were examined to assess the inhibitory effect of each bacterial isolate against *Aspergillus brasiliensis*.

2.7 Molecular Identification

Selected bacterial isolates were prepared for molecular identification and submitted to a sequencing facility for complete taxonomic analysis. Cultures of each isolate were provided by the scientist, from which genomic DNA was extracted. The quality of the extracted DNA was assessed by running it on a 1.0% agarose gel, where a single band of high-molecular-weight DNA confirmed its integrity.

To amplify the 16S rDNA gene, universal primers 27F and 1492R were used in a polymerase chain reaction (PCR). The resulting PCR product was resolved on an agarose gel, revealing a distinct amplicon of approximately 1500 base pairs, indicating successful amplification. The PCR product was then purified to remove residual primers, nucleotides, and other impurities.

Sequencing of the purified amplicon was carried out using both forward and reverse primers with the BDT v3.1 Cycle Sequencing Kit on an ABI 3730xl Genetic Analyzer. The resulting sequence data from both directions were processed using aligner software to generate a consensus sequence of the 16S rDNA gene for each isolate.

To identify the bacterial strains, the consensus sequences were subjected to BLAST analysis against the NCBI GenBank database. The top ten matches with the highest identity scores were selected for further comparison. These sequences were aligned using Clustal W multiple alignment software to assess genetic similarity. Finally, MEGA 7 software was used to construct a distance matrix and generate a phylogenetic tree, providing insights into the evolutionary relationships among the isolates and their closest known relatives.

3. Results and Discussion

A total of 10 bacterial strains were isolated from the dung of indigenous Hariana cows. In a preliminary analysis cow dung microbial load was checked and results obtained for total bacteria count (TBC) 7.4×10^5 to 7.2×10^6, total yeast and mold count (Y&MC) 3×10^3 to 4.1×10^4, total coliform count (TCC) 3.45×10^4 to 4.80×10^4. The functional analysis of these isolates revealed that 6 strains possessed zinc-solubilizing capabilities, 1 strain was phosphate-solubilizing, 4 strains were nitrogen-fixing, and 4 strains exhibited potassium-solubilizing activity. Notably, the Hariana cow dung isolates demonstrated the broadest range of activities, with one strain exhibiting all four functional properties. These observations suggested that the microbial community in Hariana cow dung was a rich source of multifunctional plant-growth-promoting bacteria that could potentially be exploited for developing biofertilizers. The detailed distribution of functional activities was summarized in (Table 1).

This suggested that the indigenous Hariana cows might harbor a more versatile microbial population with multiple soil nutrient-solubilizing and fixing capabilities. This may indicate differences in diet, genetics, or environmental conditions influencing the microbial community in these breeds.

Table 1: Analysis of dung of indigenous Hariana cows for microbiological population and activities

Total Bacteria Count, CFU/g	Total Coliforms, CFU/g	Yeast & molds, CFU/g	Isolate code	Phosphate Solubilizing, zone (mm)	Potassium Solubilizing, zone (mm)	Zinc Solubilizing, zone (mm)	Nitrogen Activity, zone (mm)
7.4×10^5 to 7.2×10^6	3.45×10^4 to 4.80×10^4	3×10^3 to 4.1×10^4	HD1	-	-	6.2	-
			HD2	-	-	9.6	-
			HD3	9.85	16.4	14.7	18.7
			HD4	-	3.6	13.5	11.6
			HD5	-	-	-	-
			HD6	-	-	-	-
			HD7	-	11.9	-	-
			HD8	-	25.5	-	11.5
			HD9	-	-	12.1	-
			HD10	-	-	11.4	13.7

3.1 Biochemical Tests of Isolates

The biochemical tests conducted on the dung samples from indigenous Hariana cows revealed distinct bacterial profiles. 8 isolates from the Hariana cow dung were identified as Gram-negative bacilli, and 2 were Gram-positive (1 Cocci and 1 Bacillus). It was observed that mainly the Gram-positive bacillus strains exhibited zinc-solubilizing activity. These findings are detailed in (Table 2).

In biochemical characterization, HD1, HD2, HD4, HD5, HD6, HD7 and HD10 were identified as Gram-negative bacillus. HD3 and HD8 were Gram-positive cocci and bacillus, respectively. All these biochemical responses of the selected strain are summarized in (Table 2).

Table 2: Biochemical tests of the bacteria isolated from Indigenous Hariana cow's dung

Isolates code	Gram Staining	Shape	Indole	Citrate	Urease	Methyl Red	Voges Proskauer	Triple Sugar Iron	Catalase	*E.coli*	Oxidase	Mannitol Salt Agar	Sucrose fermentation	H_2S	Lactose Fermentation
HD3	Positive	Cocci	-	+	-	+	-	D/L/S	+	-	-	+	+	+	+
HD8		Bacillus	+	-	+	+	-	D	+	-	+	+	+	+	+
HD1	Negative	Bacillus	-	-	-	+	-	D/L/S	-	+	-	-	-	-	+
HD2			-	+	-	+	-	D	+	+	-	-	-	-	+
HD4			+	-	-	+	-	D	-	+	-	-	-	-	+
HD5			+	-	-	+	+	D/L/S	-	-	-	-	+	-	-
HD6			+	-	-	+	-	-	-	-	-	-	-	-	-
HD7			+	-	-	+	-	D	-	+	-	-	-	-	+
HD9			+	-	-	+	-	D	+	+	-	-	-	-	+
HD10			-	+	-	+	-	D/L/S	+	+	-	-	-	-	-

D/L/S: dextrose, lactose or sucrose fermentation; D: dextrose fermentation; **(-):** Absence of carbohydrate fermentation

3.2 Evaluation of the Antagonism Effect of Bacterial Isolates

All selected bacterial isolates were co-cultured with *Aspergillus* sp. The antagonistic activity (between bacteria-fungal) here likely refers to the ability of these isolates to inhibit the growth of specific pathogens or competing microbes, a trait of significant interest in biological control and sustainable agriculture (Table 3).

Hariana cow dung isolates revealed variable antagonistic profiles. Two isolates (HD1 and HD2) exhibited strong antagonistic activity ("+++"), whereas three isolates (HD3, HD4, and HD5) did not display any detectable antagonistic effect ("-"). The remaining isolates showed moderate activity, with HD6, HD8, and HD9 registering as "++" and HD10 as "+". This distribution indicated that a subset of the Hariana cow dung isolates possesses considerable antagonistic potential, which could be harnessed for biocontrol applications, while others fall short of exhibiting a significant inhibitory effect.

Overall, the heterogeneity in antagonistic activity among bacterial isolates from Hariana cow breeds was observed. The presence of isolates with strong inhibitory properties highlighted the potential of these bacteria in developing microbial consortia aimed at the suppression of soil-borne pathogens. Such consortia could serve as an eco-friendly alternative to chemical pesticides in promoting plant health and sustainable crop production.

Table 3: Antagonistic activity of the isolates from Hariana cow dung

Isolates (Hariana)	Antagonistic activity
HD1	+++
HD2	+++
HD3	-
HD4	-
HD5	-
HD6	++
HD7	-
HD8	++
HD9	++
HD10	+

3.3 Molecular identification

Through the molecular identification process, all isolates were identified, which confirmed the strain as HC3 (*Brachybacterium paraconglomeratum*), HC4 & HC7 (*Shigella dysenteriae*), HC8 (*Bacillus safensis*), and HC10 (*Escherichia marmotae*). The phylogenetic tree were obtained and summarized in Figure 1.

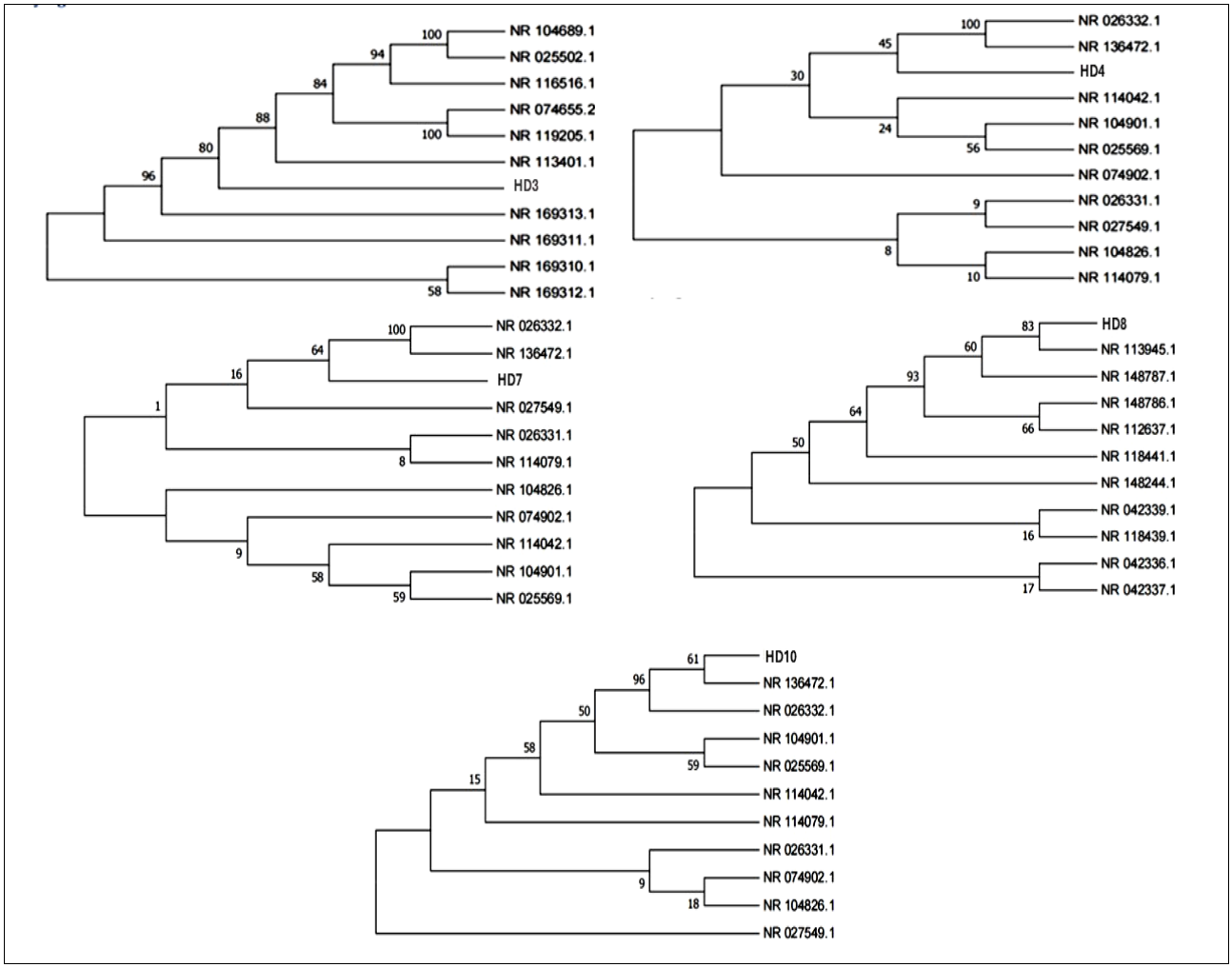

Fig 1: Phylogenetic tree of molecular identified strain

Isolates	Identified Organism	Activity showed
HD3	*Brachybacterium paraconglomeratum*	PhS, PS, ZS, NF
HD4	*Shigella dysenteriae*	PS, ZS, NF
HD7	*Shigella dysenteriae*	PS, ZS, NF
HD8	*Bacillus safensis*	PS, NF
HD10	*Escherichia marmotae*	ZS

Molecular identification was conducted for the top 5 isolates exhibiting significant functional activities such as phosphate solubilizing (PhS), potassium solubilizing (PS), zinc solubilizing (ZnS), and nitrogen-fixing (NS). The purpose of this analysis was to precisely determine the bacterial species responsible for these beneficial soil-microbe interactions and to ensure that the consortia prepared for potential agricultural applications were safe and effective. The identified organisms included a variety of species, such as *Brachybacterium paraconglomeratum*, *Shigella dysenteriae*, *Bacillus safensis*, and *Escherichia marmotae*. The identified isolates, along with their respective functional activities, were detailed in (Fig 1). To ensure the safety and efficacy of the bacterial consortia being prepared, *Shigella dysenteriae* was meticulously segregated from the non-pathogenic microorganisms. This segregation was crucial, as it prevents the inclusion of harmful pathogens in the consortia that could pose risks to plant health or human safety.

4. Discussion

A wide variety of microflora had been found in cow manure; approximately 60 bacterial species had been discovered. To screen for microorganisms with high biodegradation potential, cow dung had served as a valuable bioresource. Cow manure had proven to be a rich source of microorganisms [18]. Microbial diversity and a stable ecological balance system had been abundant in the guts of ruminant animals, especially cows.

Cow dung, primarily composed of lignin, cellulose, hemicelluloses, and 24 different minerals, had represented a biological waste product expelled by herbivorous bovines after digestion. It had harbored a vast array of microorganisms, including over 60 bacterial species and 100 species of protozoa and yeast, making it one of the richest microbial sources [19].

Microorganisms had played a vital role in preventing plant diseases and improving soil composition and health, thereby supporting plant growth and development [20]. Living microorganisms, known as biological fertilizers, had enhanced plant development by increasing mineral nutrient availability, dissolving phosphorus, and synthesizing phytohormones. Microorganisms producing plant growth regulators had benefited host plants by expanding root systems, improving nutrient and water uptake, and increasing plant resilience [21].

In the present study, several isolates had been identified, confirming the strains as HC3 (*Brachybacterium paraconglomeratum*), HC4 & HC7 (*Shigella dysenteriae*), HC8 (*Bacillus safensis*), and HC10 (*Escherichia marmotae*). *B. paraconglomeratum* had emerged as a microbe of interest due to its role in the nitrogen cycle, enzyme production, and bioremediation potential. Its versatility had demonstrated value in both industrial and environmental contexts. As an actinobacterium, *B. paraconglomeratum* had possessed the unique ability to produce polyhydroxybutyric acid (PHB)

compounds [22].

A study had reported that biohardened banana plantlets treated with *B. paraconglomeratum* and *B. amyloliquefaciens* had shown improved growth, yield, and reduced disease severity under field conditions compared to controls. These plantlets had produced higher bunch yields (13.85 kg/plant, 42.74 t/ha in plant crop; 12.55 kg/plant, 38.72 t/ha in ratoon crop) and lower disease indexes (11.1% in plant crop; 10.8% in ratoon crop) [23].

Bacillus safensis had colonized diverse habitats due to its resilience in harsh conditions and physiological traits, including tolerance to heavy metals, salt, and UV/gamma radiation. The endophytic strain TS3 of *B. safensis* had produced phytohormones, improved plant yield in field trials, and mitigated salt stress in pot experiments. It had synthesized enzymes and phytohormones such as tZ, ABA, and IAA, and had counteracted salinization effects in oat and radish plants, showing beneficial impacts of 108% and 46.7%, respectively [24].

A fertilization program using a formulation of *B. siamensis* and *B. safensis* had matched the productivity of traditional tomato fertilization, even with a 33% reduction in chemical inputs [25]. After 21 days of incubation, *B. safensis* strains had shown improved survival and higher colony-forming units in carriers. Polyethylene glycol solutions at 15% and 25% concentrations had been administered to induce drought stress six- and fifteen-days post-germination [26].

B. safensis P1.5S had demonstrated tolerance to acidic and alkaline conditions, thriving at 37°C and 15 g/L NaCl. It had released organic acids—lactic, acetic, and succinic—to solubilize phosphorus. Abiotic stressors had not affected its P-solubilization efficiency (19.54 µg P/mL), supporting its application in challenging agricultural environments [27].

In vitro PGPR tests—including phosphate solubilization, siderophore production, IAA synthesis, and ACC deaminase activity—had shown positive responses from *B. safensis* (W10) isolated from wheat rhizosphere under high sodium chloride conditions [28]. *B. safensis* PG-54 had exhibited superior plant growth-promoting traits: IAA (73.98 µg/ml), gibberellic acid (3.44 mg/ml), ammonia (37.53 µmol/ml), phosphate solubilization (88.76 µg/ml), and exopolysaccharide synthesis (1.33 g/L). Seed germination had reached 87%, with a seed vigor index of 113.1. Under salt stress, PG-54 h [ad enhanced sorghum growth metrics and biochemical indices [29].

B. safensis SCAL1 had reduced heat stress in tomato cultivars (*Solanum lycopersicum* cv. Sweetie and Riogrande). It had produced ACC deaminase (0.84-0.96 µM/mg protein/h) and EPS (0.73-0.92 mg/ml) under both normal and heat-stressed conditions. Under stress, ACC deaminase and EPS levels had increased by 12.5% and 20.65%, respectively. The strain had also generated plant growth regulators—kinetin, GA3, and IAA—under both conditions [30].

B. safensis strains isolated from cow dung had shown promise as biofertilizer candidates due to their unique agricultural capabilities. Cow excreta microbiota had also exhibited insecticidal properties, offering dual benefits of pest control and agricultural enhancement. This study had focused on utilizing underexplored cow dung microbiota for conservation and agronomic applications.

Conversely, *Shigella dysenteriae* though isolated from animal feces, had been pathogenic and not directly beneficial for agriculture [31, 32]. *B. paraconglomeratum*, *B. safensis* and *Escherichia marmotae* had emerged as promising plant growth-promoting bacteria (PGPB) due to their multifaceted roles in enhancing plant health and productivity.

5. Conclusion

In conclusion, dung from indigenous Hariana cows is a rich reservoir of beneficial microbes that play a pivotal role in nutrient solubilization, thereby enhancing the efficiency of bio-compost derived from this resource. Microbial analysis of Hariana cow dung revealed the presence of key bacterial populations capable of solubilizing zinc, phosphate, and potassium, along with exhibiting nitrogen fixation activity. Notable identified species included *Brachybacterium paraconglomeratum*, *Shigella dysenteriae*, and *Bacillus safensis*, among others. Isolates demonstrating multiple functional traits—namely zinc, phosphate, and potassium solubilization, coupled with nitrogen fixation—were particularly significant for their potential application in sustainable agriculture. These multi-activity strains offer dual benefits: mobilizing essential nutrients and enriching soil nitrogen content, which collectively reduce the reliance on synthetic fertilizers. By actively decomposing organic matter and releasing bioavailable nutrients, these microbes contribute to improved plant growth and enhanced soil health. The findings not only highlight functional diversity among microbial communities associated with different cow breeds but also emphasize the untapped potential of indigenous cow dung as a source of plant-growth-promoting bacteria. Harnessing this microbial richness could be instrumental in developing biofertilizers tailored to improve soil fertility and support ecologically sound farming practices. The integration of such native microbial consortia into agricultural systems represents a promising step toward long-term sustainability and reduced chemical input.

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