**Isolation and Characterization of Multifunctional Plant-Growth-Promoting Bacteria from Indigenous Sahiwal Cow Dung for Sustainable Agriculture**

**Abstract**

India, with its rich livestock diversity, has indigenous cow breeds such as Sahiwal. These native breeds have evolved resilience to climatic stress and nutritional scarcity, yet remain underutilized due to low milk yield. This study explores an alternative valorization strategy by investigating the microbial potential of Sahiwal cow (from Sonipat District of Haryana) dung for potential agricultural applications. Fresh samples were analyzed for microbial load and then subjected to the isolation, identification, and functional characterization of the associated microflora. Particular focus was placed on plant-growth-promoting attributes such as nutrient solubilization (zinc, potassium, phosphate), nitrogen fixation, and antagonistic activity. The findings reveal a diverse bacterial consortium with significant functional traits, highlighting the promise of cow dung-derived microbial consortia in sustainable farming. By linking indigenous biodiversity with organic soil enrichment practices, this work underscores the dual benefit of breed conservation and eco-friendly agricultural productivity.

**Keywords:** Indigenous cows, Sahiwal cow dung, Nutrient solubilization Activities, Plant-Growth-Promoting Bacteria,Antagonistic activity

1. **INTRODUCTION**

India is home to over 193.46 million cattle, accounting for more than 36% of the nation's total livestock and approximately 14.5% of the global cow population (DAHD, 2022). Cattle have historically played a vital role in Indian agriculture, significantly contributing to livelihoods, cultural traditions, and global food security (Kolekar et al., 2023). Among these, indigenous cattle constitute 142.11 million, representing 73.45% of India’s cow population. With 53 recognized breeds (ICAR, 2024), India holds the highest diversity of cow breeds in the world. Indigenous breeds are naturally adapted to their local environments, having evolved over generations within specific breeding tracts. These breeds exhibit resilience to high temperatures, resistance to endemic diseases, and the ability to thrive under nutritional stress. Traditionally, they have served multifunctional roles in rural households, including milk production, draught power, and manure contribution (Bhandari et al., 2021). Their adaptive mechanisms to harsh environments—manifested through physiological, morphological, behavioral, neuroendocrine, and biochemical traits—enhance their capacity to withstand climate change (Sarang et al., 2024). According to the IPCC (2022), global temperatures are projected to rise beyond 1.5°C between 2021 and 2040. In this context, conserving and promoting climate-resilient breeds becomes imperative. Institutions such as the ICAR-National Bureau of Animal Genetic Resources (ICAR-NBAGR) are executing time-bound action plans for surveying, characterizing, and identifying conservation priorities for these native breeds (Srivastava et al., 2019).

Despite their value, indigenous cows are often underutilized due to low milk yield or non-reproductive status with advancing age. As a result, they are frequently sold to traders, abandoned near roadsides, or left in forested areas (Pun et al., 2024). To reverse this trend, it is essential to explore alternative value streams that can make these cattle economically viable to farmers, thereby incentivizing their preservation. One such avenue lies in the microbial richness of indigenous cow dung. These cattle, due to their adaptive physiology, harbor a uniquely enriched microbiota in their dung and urine. Exploring this native microbial population offers significant potential in organic farming. Indigenous cow dung can serve as a source for microbial consortia with zinc-, potassium-, and phosphate-solubilizing abilities, nitrogen fixation potential, and pest-suppressive properties. As such, bio-formulations developed from this resource can act as sustainable alternatives to chemical soil inoculants and biopesticides (Sagar et al., 2024). Cattle in India, particularly cows, produce 10–20 kg of dung per day, providing a steady reservoir of beneficial microbes. Dung-borne bacteria are known to promote plant growth and enhance plant resilience to diseases. Its incorporation into soil improves biomass productivity by encouraging the growth of symbiotic bacteria and enhancing nutrient and organic matter availability (Dhiman et al., 2021). Vedic literature even regards cow dung (Gomeya) as a sacred and purifying substance, reinforcing its cultural and ecological relevance.

Cow dung contains approximately 60 bacterial species, primarily from the genera Bacillus, Corynebacterium, and Lactobacillus, alongside fungal species like Aspergillus and Trichoderma, nearly 100 protozoan species, and several yeasts. Studies have identified additional bacterial genera such as Alcaligenes, Serratia, and Acinetobacter (Bhatt and Maheshwari, 2019). A recent study also reported the presence of Aspergillus niger, Aspergillus fumigatus, and 20 bacterial isolates, including E. coli, Micrococcus sp., and Bacillus sp. (Devi et al., 2023). Vermicompost prepared using cow dung has been shown to improve soil texture, water retention capacity, organic carbon levels, and microbial diversity, thereby enhancing crop yields (Banerjee et al., 2023). Such practices not only promote organic productivity but also generate additional value from indigenous cattle through eco-friendly by-products. Utilizing enriched biocompost derived from native cow dung presents an opportunity to mitigate environmental degradation while increasing soil fertility, farm income, and sustainability (Behera et al., 2021). Biofertilizers derived from dung microflora may also serve in bioprotection against plant pathogens, encouraging a transition toward biological farming practices, particularly for farmers interested in organic, sustainable agriculture (Yadav et al., 2025).

To harness this potential, it is vital to analyze the microbial profile of fresh cow dung and urine through isolation, identification, and functional characterization. In parallel, composting trials can assess the durability and microbial enrichment of dung-based biocompost. In this study, the Sahiwal indigenous breed was selected to investigate the nutrient solubilization and antagonistic potential of its microbial community. The experimental work involved the isolation and identification of microbial strains using biochemical and molecular methods and the evaluation of their functional properties, including antagonism, solubilization of zinc, potassium, and phosphate, and nitrogen fixation activity.

1. **MATERIALS AND METHODS**
   1. **Sample Collection**

Fresh sahiwal cow dung samples from the mid part of the excreted dung are collected in a sterile polybag. Collected samples are processed for analysis within 24 hrs of collection to avoid the saturation of the sample.

### **2.2. Media Preparation & Sample Inoculation**

The media used for preliminary processing included Plate Count Agar (PCA), Chloramphenicol Yeast Glucose Agar (CYGA), Violet Red Bile Agar (VRBA), and Minimum Recovery Diluent (MRD) as the diluent. The media, except for VRBA, were autoclaved at 121°C and 15 PSI for 15 minutes. The VRBA medium was only boiled and subsequently poured.

For sample preparation, 10 g of dung and 10 ml of urine were added to 90 ml of MRD, followed by serial dilution for the isolation process. After completing the serial dilution, 1 ml of each dilution in duplicate was transferred to plates for Total Bacterial Count (TBC), Total Yeast and Mold Count (Y&MC), and Total Coliform Count (TCC) estimation. Using the pour-plate method, autoclaved PCA, CYGA, and boiled VRBA were poured into sample-containing plates labelled between 45-50°C, ensuring proper mixing by rotating clockwise and counterclockwise to avoid spillage. The plates were left to solidify. PCA and CYGA plates were incubated at their optimal temperatures of 30°C and 25°C for 3 and 5 days, respectively, for overall microbial growth. In the case of VRBA, after solidification, 4 ml of VRBA was added to each plate, mixed, and left to solidify before incubation at 37°C for 24 hours.

**2.3. Isolation and identification**

VRBA plates were examined for total coliform count (TCC) estimation after 24 hours of incubation, and the results were recorded. Plates containing PCA and CYGA media for both dung and urine samples were inspected after 3 days, revealing bacterial colonies with distinct morphological variations, which were then selected for further characterization. For yeast and mold growth, CYGA plates were evaluated after 5 days of incubation. Fungal colonies, differentiated by their morphological variations, were picked from these plates. Additionally, Total Bacterial Count (TBC) and Total Yeast and Mold Count (Y&MC) for both dung and urine samples were assessed from these plates.

**2.4.** **Strain Characterization**

A total of 11 bacterial isolates were isolated and selected for strain characterization**.** Selected strains upon morphological characteristics and variations are checked for qualitative zinc, potassium, phosphate solubilization, and nitrogen fixation activity as the primary concern of the research associated with the agriculture-friendly microbial consortia development. After the activity check-up, beneficial activity and positive strains were further evaluated for biochemical characterization, antagonistic activity and molecular characterization for identification.

**2.4.1 Zinc Solubilizing Activity**

The zinc solubilizing activity was assessed using Zinc Solubilizing Agar (Himedia-M2068), which contains dextrose (glucose), ammonium sulfate, potassium chloride, dipotassium hydrogen phosphate, magnesium sulfate heptahydrate, zinc oxide, and agar. The pH of the medium was maintained at 7.2, and it was autoclaved at 121°C for 15 minutes at 15 PSI. Selected bacterial isolates were streaked on the solidified, contaminant-free media plates and incubated at 30°C for 2 days, or 4-5 days for slow-growing isolates, to observe zinc solubilizing activity. Zinc-solubilizing strains exhibited a clear zone surrounding the colony. The measurement of this halo zone indicated the solubilizing activity (Srithaworn et al., 2023).

**2.4.2 Potassium Solubilizing Activity**

Potassium solubilizing activity was assessed using Aleksandrow agar (Himedia-M1996), which contains dextrose (glucose), magnesium sulfate, ferric chloride, calcium carbonate, calcium phosphate, and potassium alumino-silicate. The medium was maintained at pH 7.2 and autoclaved at 121°C for 15 minutes at 15 PSI. Selected bacterial isolates were streaked on solidified, contaminant-free media plates and incubated at 28°C for 7 days to observe potassium solubilizing activity. The halo zone surrounding the colonies was measured as an indicator of potassium-solubilizing activity after 7 days of full growth (Sood et al., 2023).

**2.4.3 Phosphate Solubilizing Activity**

Phosphate-solubilizing activity was evaluated using Pikovskaya’s agar (Himedia-M520), which contains yeast extract, dextrose (glucose), magnesium sulfate, potassium chloride, calcium phosphate, ammonium sulfate, manganese sulfate, and ferrous sulfate. The medium was maintained at pH 7.2 and autoclaved at 121°C for 15 minutes at 15 PSI. Selected bacterial isolates were streaked on solidified, contaminant-free media plates and incubated at 37°C for 5 days to observe phosphate solubilizing activity. The halo zone surrounding the colonies was measured as an indicator of phosphate solubilizing activity after 5 days of full growth (Agboola et al., 2023).

**2.4.4 Nitrogen Fixation Activity**

Nitrogen fixation activity was assessed using Norris glucose nitrogen-free medium (Himedia-M712), which contains dextrose (glucose), dipotassium hydrogen phosphate, magnesium sulfate, calcium carbonate, sodium chloride, sodium molybdate, and ferrous sulfate. The medium was maintained at pH 7.0 and autoclaved at 121°C for 15 minutes at 15 PSI. Selected bacterial isolates were streaked on solidified, contaminant-free media plates and incubated at 28°C for 7 days to observe nitrogen fixation activity. Nitrogen fixation activity was assessed by measuring the halo zone surrounding the colonies 7 days after complete development (Zhang et al., 2022).

**2.4.5 Biochemical Characterization**

A total of 15 different biochemical tests were conducted on selected dung bacterial isolates after initial activity screening. Each bacterial isolate was inoculated and incubated overnight at 37°C. The overnight-grown cultures were analyzed for Gram staining, visualized under a compound microscope at 100x magnification, to determine bacterial morphology and Gram status (positive or negative). The cultures were then subjected to catalase and oxidase testing. The oxidase test identifies microorganisms that produce cytochrome oxidase, which facilitates electron transfer from the electron transport chain to the final acceptor (oxygen), resulting in water formation (Shoaib et al., 2020). The oxidase test was performed by soaking filter paper with 1% tetramethyl-p-phenylenediamine dihydrochloride (an artificial electron donor) and drying the paper. Bacterial colonies were spread onto the paper strip, and the color change was monitored for 10 seconds, with a dark purple shift indicating oxidase activity. The presence of catalase, an enzyme that catalyzes the decomposition of hydrogen peroxide (H2O2) into water and oxygen, was detected using 3% H2O2. This test distinguishes bacteria that produce the catalase enzyme (Shoaib et al., 2020; Syahri et al., 2019).

In addition, each culture was inoculated into various media for further biochemical tests, including peptone for the indole test; Methyl Red Voges-Proskauer (MRVP) media for Methyl Red and Voges-Proskauer tests; lactose broth for the lactose test; nutrient broth for bubble formation; urease media for the urease test; Eosin Methylene Blue Agar (EMB) for Gram-negative bacterial isolation; Mannitol Salt Agar (MSA) for Gram-positive bacterial isolation; citrate media for the citrate utilization test; and triple sugar iron agar for the confirmation of dextrose, lactose, sucrose, and H2S formation. All inoculated media were incubated at 37°C for 24 hours.

**2.4.6 Molecular Identification**

For comprehensive identification, the selected bacterial isolates (SD5, SD6, SD7, SD11, SU3, SU6, and SU7) were prepared for molecular identification and sent to a sequencing centre. DNA was isolated from the cultures, and its quality was evaluated on a 1.0% agarose gel, revealing a single band of high-molecular-weight DNA. A fragment of the 16S rDNA gene was amplified using 27F and 1492R primers, resulting in a single discrete PCR amplicon band of approximately 1500 bp, as observed on the agarose gel. The PCR amplicon was purified to remove contaminants, and forward and reverse DNA sequencing reactions were performed using the BDT v3.1 Cycle Sequencing Kit on an ABI 3730xl Genetic Analyzer. A consensus sequence of the 16S rDNA gene was generated from the forward and reverse sequence data using the aligner software. The 16S rDNA gene sequence was used to conduct a BLAST search against the NCBI GenBank database. Based on the maximum identity score, the top ten sequences were selected and aligned using the Clustal W multiple alignment software program. A distance matrix was generated, and a phylogenetic tree was constructed using MEGA 7.

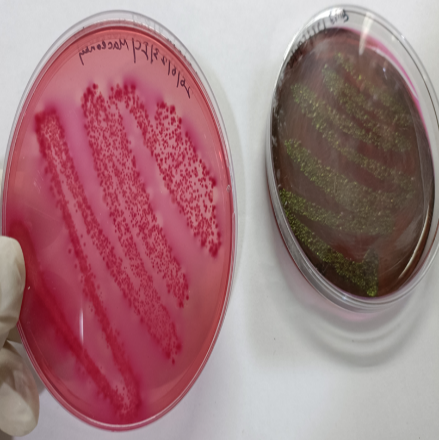
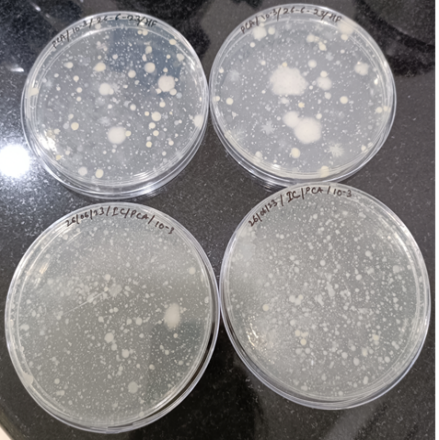
1. **Results & Discussion**

3.1 Isolation of Bacteria from Cow Dung and Analysis of Nutrient Solubilizing Activities

A total of 11 distinct bacterial strains were isolated from the dung of indigenous Sahiwal cows. Functional analysis of these isolates revealed a broad range of plant-growth-promoting properties. Specifically, six strains demonstrated zinc solubilization, four exhibited potassium solubilizing activity, and two showed nitrogen-fixing ability. However, none of the strains displayed phosphate solubilization capacity.

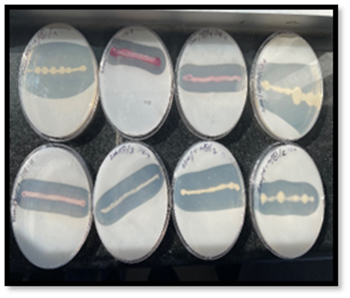
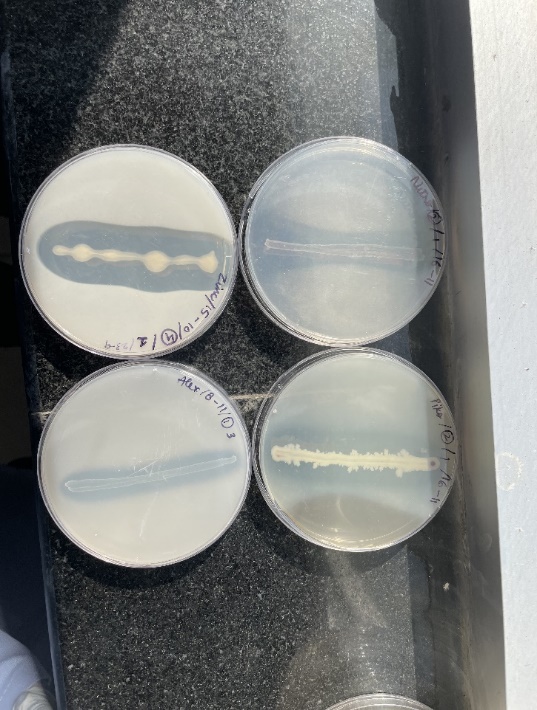
In a preliminary assessment of microbial load in Sahiwal cow dung and urine, the total bacterial count (TBC) ranged from 3.3 × 10⁵ to 6.68 × 10⁶ CFU/g in dung. The total yeast and mold count (Y&MC) varied between 3.1 × 10⁴ and 7.27 × 10⁴ CFU/g, while the total coliform count (TCC) ranged from 3.4 × 10⁴ to 2.77 × 10⁵ CFU/g.

Zinc solubilization was demonstrated by isolates SD5 (4.4 mm), SD6 (5.4 mm), SD11 (4.3 mm), and SU6 (14.09 mm). Potassium solubilization was observed in SD6 (6.7 mm), SU3 (15.40 mm), SU6 (15.32 mm), and SU7 (20.58 mm). Nitrogen fixation activity was exhibited by SU3 (29.65 mm) and SU7 (36.70 mm). None of the isolates produced zones indicating phosphate solubilization (Fig. 1, Table 1).



(a)

(b)

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(d)

(c)

**Fig. 1 (a)** Bacteria isolated from the indigenous cow dung, **(b)** bacterial growth on selective media, **(c, d)** Clear zone shown by the bacterial isolates showing Zinc, potassium solubilizing and nitrogen fixation activity

**Table 1:** Analysis of the dung of Sahiwal cows for microbiological population and activities

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **S/N** | **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)** |
| 1 | 3.3 x 105 to 6.68 x 106 | 3.1 x 104 to 7.27 x 104 | 3.4 x 104 to 2.77 x 105 | SD1 | - | - | - | - |
| 2 | SD5 | - | - | 4.4 | - |
| 3 | SD6 | - | 6.7 | 5.4 | - |
| 4 | SD7 | - | - | 4.2 | - |
| 5 | SD8 | - | - | 3.8 | - |
| 6 | SD9 | - | - | - | - |
| 7 | SD10 | - | - | 4.3 | - |
| 8 | SD11 | - | - | - | - |
| 9 | SU3 | - | 15.40 | - | 29.65 |
| 10 | SU6 | - | 15.32 | 14.09 | - |
| 11 | SU7 | - | 20.58 | - | 36.70 |

**3.2 Biochemical characterization**

Biochemical characterization revealed that SD5, SD6, SD7, SD11, SU6, and SU7 were Gram-positive rods, while SU3 was identified as a Gram-negative rod. All strains tested negative for indole and urease but were positive for mannitol fermentation. Catalase activity was observed in all isolates except SD7. The Voges-Proskauer test was positive only for SD11, whereas citrate utilization was detected in SD5, SD6, SD11, SU3, and SU6. The methyl red test was positive for SD5, SD6, SD7, and SU7. Hydrogen sulfide (H₂S) production was recorded in SD6, SU6, and SU7.

Triple sugar iron (TSI) test results indicated dextrose fermentation in SD5, SD7, SD11, SU3, and SU6. Isolate SU7 was capable of fermenting dextrose, lactose, or sucrose. In contrast, SD6 showed no fermentation activity in the TSI test.

These findings highlight the functional diversity of the microbial population in Sahiwal cow dung. The presence of multiple strains with nutrient-solubilizing and nitrogen-fixing capabilities underscores the potential of this microbial consortium as a source of multifunctional biofertilizer candidates. Despite the absence of phosphate solubilization, the results suggest that indigenous Sahiwal cows host a microbiota with promising applications in sustainable agriculture. The detailed distribution of functional traits is summarized in Table 2, and corresponding activities are illustrated in Figure 2.

**Table 2:** Biochemical characterization chart for selected strains

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Isolates code** | **Gram Staining** | **Shape** | **Indole** | **Citrate** | **Urease** | **Methyl Red** | **Voges Proskauer** | **Triple Sugar Iron** | **Catalase** | ***E. coli*** | **Oxidase** | **Mannitol Salt Agar** | **Sucrose Fermentation** | **H2S** | **Lactose Fermentation** |
| SD5 | Positive | Bacilli (rods) | - | + | - | + | - | D | + | - | + | + | - | - | - |
| SD6 | - | + | - | + | - | - | + | - | + | + | - | + | - |
| SD7 | - | - | - | + | - | D | - | - | + | + | - | - | - |
| SD11 | - | + | - | - | + | D | + | - | + | + | - | - | - |
| SU6 |  |  | - | + | - | - | - | D | + | - | + | + | + | + | - |
| SU7 | - | - | - | + | - | D/L/S | + | - | - | + | + | + | + |
| SU3 | Negative | - | + | - | - | - | D | + | - | - | + | - | - | - |
| # **D/L/S:** dextrose, lactose, or sucrose fermentation; D: dextrose fermentation; **(-):** Absence of carbohydrate fermentation | | | | | | | | | | | | | | | |

**3.3 Antagonistic activity**

The antagonistic potential of selected bacterial isolates from Sahiwal cow dung was assessed against Aspergillus brasiliensis, a fungal phytopathogen of concern in agriculture and food safety, using a qualitative scoring system. The activity was denoted as strong (+), moderate (), weak (+), or absent (–), depending on the observable zone of inhibition. The assay results revealed differential antifungal activity among the isolates, categorized based on the extent of mycelial growth inhibition. Isolates **SD5, SD6, and SU3** exhibited **strong (+++) antagonistic activity,** indicating their potential to produce robust antifungal metabolites or engage in effective mycoparasitism or competitive exclusion. Isolates **SD7, SD11, SU6, and SU7** demonstrated **moderate (++) inhibition**, suggesting the presence of suppressive traits, albeit with relatively reduced efficacy compared to the strongly active strains (Table 3). These findings indicate that multiple bacterial isolates from Sahiwal cow dung possess noteworthy antagonistic properties against A. brasiliensis, highlighting their potential application in the development of bio-based fungicidal formulations. Their activity implies the potential production of antimicrobial metabolites or competitive colonization ability, contributing to pathogen suppression in the rhizosphere. Such antagonism may be attributed to mechanisms like secondary metabolite production, secretion of lytic enzymes, or siderophore-mediated competition, warranting further molecular investigation.

**Table 3: Antagonistic activity of selected strain**

|  |  |  |
| --- | --- | --- |
| **Isolates** | **Antagonistic activity** |  |
| SD5 | +++ |
| SD6 | +++ |
| SD7 | ++ |
| SD11 | ++ |
| SU3 | +++ |
| SU6 | ++ |
| SU7 | +++ |

**3.4 Molecular Identification**

Through the molecular identification process, all isolates were identified, which confirms the strain as SD5 (*Bacillus stercoris*), SD6 (*Priestia megaterium*), SU7 (*Bacillus stratosphericus*), SD11 (*Bacillus aerius*), SU3 (*Enterobacter hormaechei*), and SU6 (*Lysinibacillus boronitolerance*). SU7 showed mixed readings, so it was taken for further experiments. The phylogenetic tree was obtained and summarized in Fig. 2.

|  |  |
| --- | --- |
|  |  |
|  |  |
|  |  |

**Fig. 2:** Phylogenetic tree of molecularly identified strains

1. **Discussion**

In India, cow dung and urine have long been utilized as natural insecticides and fertilizers, and recent studies have shown how well they work to support sustainable agriculture. Organic matter, vital elements like potassium, phosphorus, and nitrogen, and helpful microbes that can improve soil fertility and plant growth can all be found in abundance in cow dung. Conversely, cow urine is a powerful fertilizer and insect repellent due to its high concentrations of nitrogen, urea, and minerals (Patra and Bharti, 2024). Cow dung is the most important source of biofertilizer and is used to generate power in many developing countries. It is a very effective alternative to artificial fertilizers, which eventually increase productivity, maintain soil health, and promote microbial populations. Cow dung and vermicompost increase soil organic matter, which improves water penetration and retention as well as cation exchange capacity. Cow dung and urine include a variety of substances that are used in agriculture as fertilizer and to manage pests. In current research, molecularly identified strains were SD5 (*Bacillus stercoris*), SD6 (*Priestia megaterium*), SD7 (*Bacillus stratosphericus*), SD11 (*Bacillus aerius*), SU3 (*Enterobacter hormaechei*) and SU6 (*Lysinibacillus boronitolerans*). Strong scientific support for the biocontrol actions of *B. stercoris* strain B.PNR1 against *Fusarium* wilt in tomatoes has been provided by a recent study. Under greenhouse conditions, B.PNR1 demonstrates actions such as the production of hydrolytic enzymes and antibacterial substances (antimicrobial activity) (Ku et al., 2024; Chouaia et al., 2024), and the capacity to regulate disease. It also exhibits capacities in phosphate solubilization and IAA synthesis, which promotes improved plant growth (Pengproh et al., 2023). *Bacillus stercoris* TY-12 was also reported to treat capsicum that had been infected by *R. solanacearum*. In crop production, TY-12 decreases infection by 84.18% and may be employed as a new biocontrol microbial strain consisting of antimicrobial properties (Wang et al., 2025). P. megaterium is capable of functioning as a biocontrol (Li et al., 2022) or biopesticide. There are several different anti-pathogenic mechanisms in P. megaterium. Possible links exist between the synthesis of iron-chelating siderophores and an antifungal effect against the tea disease Fomes lamaoensis, which causes brown root rot (Biedendieck et al., 2021). Using secretory acid phosphatase and phytases, *P. megaterium* produces organic acids that serve as the primary building block for phosphate and zinc biofertilization and solubilization (Mahmoud et al., 2024). Furthermore, plants can receive reduced nitrogen from *P. megaterium*. Many *P. megaterium* fertilizer compositions, frequently in combination with other bacteria, are currently offered commercially by various producers for use in large-scale agricultural applications (Hu et al., 2013; Singh et al., 2020). *P. megaterium* (BP-R2) is a potential bioinoculant as it can decrease stress to enhance plant growth in salt and drought stress conditions (Hwang et al., 2022). It was found that when maize seedlings were pretreated with a bacterial consortium consisting of *Staphylococcus succinus* and *Bacillus stratosphericus*. This bacterial pretreatment enhanced plant growth and partially restored the root architecture system under saline stress (Oliva et al., 2023). A strain of *B. stratosphericus* that was isolated from mine contaminants has the potential to be used in the development of biopesticides to suppress bacterial phytopathogens, showing strong antibacterial activity that worked against five bacterial phytopathogens. Significant activation of the PAL gene was seen in tomato plant leaves treated with *B. stratosphericus* FW3 (Durairaj et al., 2017). According to a study, *Bacillus stratosphericus* LW-03 was isolated from *Lilium wardii* bulbs. Excellent antifungal activity was demonstrated by the isolated endophytic strain LW-03 against common plant diseases, including *Fusarium oxysporum*, *Botryosphaeria dothidea*, *Botrytis cinerea*, and *Fusarium fujikuroi*. Based on this study, *Bacillus stratosphericus* LW-03 plays a significant role in the development of biological fertilizers and sustainable biological control methods for agriculture (Zhang et al., 2022). Several *Bacillus* species are found on the market as phytostimulants, biopesticides, and biofertilizers. Because of this diverse functionality, the Bacillus genus is among the most commonly utilized in the agro-biotech sector (Etesami et al., 2023). *B. aerius* acts as a promising biocontrol agent against *B. alexandrina* snails (Saleh et al., 2022). Recent findings reveal that *B. aerius* is an excellent option as a biocontrol agent to be used by the agriculture sectors to have a sustainable environment (Malvi et al., 2022; Srivastava et al., 2024). In a study, Enterobacter hormaechei Z129, isolated from a dairy cow that contains urease, could utilize urea nitrogen (Zhong et al., 2023). It is also found that [*Enterobacter*](https://www.sciencedirect.com/topics/immunology-and-microbiology/enterobacter) *hormaechei* Wu15 in combination with [*Bacillus subtilis*](https://www.sciencedirect.com/topics/immunology-and-microbiology/bacillus-subtilis) SL44 possibly reduces the severity of the disease by increasing the proliferation of beneficial bacteria on the mycelial surface and decreasing the density of *Colletotrichum gloeosporioides* and *Rhizoctonia solani* (Wang et al., 2024). To improve seed germination, early vegetative growth of seedlings, and ultimately the yield component, Enterobacter hormaechei 40a may be a viable option for a P and K solubilizing biofertilizer for plant growth promotion (Roslan et al., 2020)*.* Comparing *Enterobacter hormaechei*-treated tomato seeds (*Lycopersicum esculentum*) to the control, the former showed increased biomass and shoot length. In addition to improving plant development, it changed the architecture of the roots, which increased crop productivity (Ranawat et al., 2021). *Lysinibacillus* species are motile, gram-positive bacteria that produce spores. The ability of *Lysinibacillus* species to promote plant development, bioremediate, act as an entomopathogen, and biologically control disease is drawing the attention of researchers (Ahsan and Shimizu, 2021). Under greenhouse conditions, twelve strains of *Lysinibacillus* spp. were tested; six of these strains improved the biomass and root structure of maize plants. The majority of the time, growth stimulation was visible at an inoculum concentration of 108 CFU/mL. The amount of IAA generated by each strain varied greatly (20–70 µg/mL)(Pantoja et al., 2023). *Lysinibacillus boronitolerance* strain has lots of potential and may be applied for agriculture to examine its zinc, phosphate, potassium, and nitrogen activity. Cow dung is a source of inoculum for helpful microorganisms. Bioformulations made from the products of native cows can provide sustainable and environmentally friendly substitutes for soil inoculants and biopesticides since cow dung contains advantageous microorganisms (Sagar et al., 2024). Modern scientific tools have been used to conduct investigations on the profound microbial diversity of cow dung, and the results offer a logical understanding of the microorganisms' potential for synergy. The extensive usage of compost (dung manure) in Indian agriculture suggests that it has the potential to boost crop yields (Sharma et al., 2022). Due to its abundance of nutritional elements (potassium, phosphorus, and nitrogen), cow dung is regarded as a useful source of fertilizer (Zhou et al., 2023). In tropical agriculture, thermotolerant *B. subtilis* strains can be effectively used as a bio-inoculant or cow dung amendment to solubilize P and preserve soil health, which is helpful in the context of global warming (Swain et al., 2012). Agricultural soils are adversely affected by chemical plant protection agents, which alter the physical characteristics of the soil (such as its texture, permeability, and porosity), disrupt the nitrogen and phosphorus cycles, and reduce the complexity of the soil microbiome (Antoszewski et al., 2022). Plant growth-promoting microorganisms, or PGPMs, are microbial bioinoculants made up of dormant or live microbes that can stimulate plant growth and development. They have enormous promise for both improving plant production and cleaning up degraded soils. In agriculture, bioinoculants are economical and environmentally beneficial methods (Basu et al., 2021; Maitra et al., 2022).

*Enterobacter cloacae*, *Klebsiella pneumoniae*, *Serratia marcescens*, and *Bacillus thuringiensis*, commonly found in rice rhizospheres, produce low-molecular-weight organic acids (gluconic, citric, oxalic) that chelate insoluble zinc compounds (ZnO, Zn₃(PO₄)₂) and acidify the rhizosphere, converting them into plant-available Zn²⁺. Many ZSB also produce siderophores that bind zinc ions and indole-3-acetic acid (IAA), which promotes root growth and nutrient uptake. In field studies on Inceptisol paddy soils, a consortium of indigenous ZSB boosted rice grain zinc content by 16.3% and yield by 5.6% above uninoculated controls (Pratiwi et al. 2024).

*Serratia marcescens* (HPY10), *Bacillus circulans*, *B. edaphicus*, *B. mucilaginosus*, and *Paenibacillus* spp. are important native potassium-solubilizing microorganisms (KSM) found in volcanic and sedimentary soils. Bacteria emit organic acids (tartaric, citric, and oxalic) that chelate K⁺ from feldspars and micas. They may also outflow exopolysaccharides and metal-complexing ligands to release potassium ions. Many KSM co-produce IAA and siderophores, which promote root development and increase disease resistance. Inoculation with native KSM has been demonstrated to increase soil-available potassium, root uptake, plant height, and grain production in both ideal and stress situations (Etesami et al. 2017; Sharma et al. 2024).

Native diazotrophs include both free-living and symbiotic bacteria. Nitrogen fixers, such as *Azotobacter* spp. (aerobic, neutral-alkaline soils) and *Beijerinckia* spp. (acid-tolerant), use the nitrogenase enzyme to convert atmospheric N₂ to NH₃ at rates of approximately 20-30 µmol N₂·h⁻¹·mg⁻¹ protein. Heterocystous cyanobacteria (such as Oscillatoria and Anabaena) colonize wet soils, enriching them with bioavailable nitrogen. Symbiotic fixers include rhizobia (*Bradyrhizobium*, *Sinorhizobium*) that develop root nodules on legumes and Frankia spp. that nodulate actinorhizal plants, supplying up to 200 kg N·ha⁻¹·yr⁻¹ and significantly lowering synthetic nitrogen fertilizer requirements (Bhat et al. 2015; Aasfar et al. 2021).

Zinc- and potassium-solubilizing bacteria mobilize micronutrients via acidification and chelation processes, but they lack nitrogen-fixing capabilities. In contrast, diazotrophs give macronutrient nitrogen without solubilizing Zn or K. Crops can be co-inoculated with customized consortia of ZSB, KSM, and diazotrophs to enhance multi-nutrient uptake, increase endogenous phytohormone levels, and improve soil health. Integrated biofertilizer strategies reduce chemical fertilizer inputs while increasing agricultural output and supporting sustainable intensification goals.

1. **Conclusion**

From the dung of indigenous cows (Sahiwal), a total of 11 bacterial strains were isolated. Indigenous Sahiwal cows' dung demonstrated a screened bacterial population of essential microbes required for zinc and potassium solubilization and nitrogen fixation. The identified organisms included a variety of species, i.e., *Enterobacter hormaechei*, *Lysinibacillus boronitolerans*, *Bacillus stratosphericus*, *Bacillus stercoris*, *Priestia megaterium*, and *Bacillus aerius*. The multi-activity isolates from the Sahiwal cow dung were particularly noteworthy for their potential applications in sustainable agriculture, offering combined benefits of nutrient solubilization and nitrogen fixation that can reduce dependency on chemical fertilizers. These observations not only highlighted the microbial functional traits but also underscored the potential of utilizing indigenous cow dung as a reservoir for plant-growth-promoting bacteria. This could be significant for developing biofertilizers aimed at enhancing soil fertility and promoting sustainable agricultural practices.

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