Assessment of Cellulolytic, Potassium, and Phosphate Solubilizing of Bacteria from Bali Cattle Rumen as Potential Compost Bioactivators

.

ABSTRACT

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| Rumen fluid of Bali cattle contains a highly diverse and functionally active bacterial community, offering valuable potential for compost bioactivation. This study aimed to isolate and characterize cellulolytic, phosphate, and potassium-solubilizing bacteria from the rumen and assess their roles in supporting composting processes. A range of bacterial isolates was successfully obtained and grown on selective media, revealing distinct functional groups including those capable of degrading cellulose, solubilizing phosphate and potassium, fixing nitrogen, and producing IAA. Functional screening using clear zone assays confirmed enzymatic activities related to organic matter decomposition and nutrient solubilization. Several isolates exhibited multifunctional traits, demonstrating simultaneous activity across multiple biochemical processes. These findings highlight the ability of rumen-derived bacteria to accelerate composting, enhance nutrient availability, and improve the overall compost quality. Compared to previous studies, the microbial diversity observed in Bali cattle rumen was notably higher, likely influenced by forage-based diets and the adaptive conditions of the rumen environment. The results support the application of these indigenous bacterial strains as effective bioactivators in sustainable composting systems and organic waste management strategies. |

*Keywords: Bali cattle, rumen fluid, cellulolytic bacteria, potassium decomposing bacteria, phosphate decomposing bacteria.*

1. INTRODUCTION

The increasing accumulation of organic waste (OW), particularly from agricultural and livestock productions, poses significant environmental and economic challenges. Sustainable management of these waste streams is crucial for promoting nutrient cycling, improving soil health, and contributing to a circular economy (Kiyasudeen et al., 2016). Currently, composting stands out as an effective and environmentally friendly method for converting OW into valuable soil amendments, aligning with broader goals of sustainable development and the Sustainable Development Goals (SDGs), particularly those related to a sustainable economy, environmental conservation, and social welfare (Senadheera et al., 2024). The composting process, while natural, can be significantly accelerated and optimized through the application of bio-activators. Rumen contents, a type of livestock waste, offer a promising and cost-effective source of bio-activators (Kiyasudeen et al., 2016). The utilization of local microorganisms (MOL) as bio-activators offers an effective and accessible solution for enhancing organic matter decomposition (Dewilda et al., 2023). MOL, defined as a collection of locally cultivated microorganisms embodying a "zero-waste" concept, can serve as an effective starter culture for organic compost production and, importantly, contribute to feed quality enhancement (Costello, 2019; Ullah et al., 2022). MOL solutions are rich in macro and micro-nutrients and contain diverse bacterial populations with multifaceted potentials. These bacteria act as effective decomposers of organic matter, plant growth stimulants, and agents for controlling pests and diseases, enabling MOL to function as both a biofertilizer enhancer and an organic biopesticide, particularly as a fungicide. Research indicates that the incorporation of MOL into composting processes results in high nutrient content in the final compost, often accompanied by a dominant cellulolytic bacterial population (Kawalekar, 2013). Consequently, MOL shows substantial potential for enhanching the quality of various agricultural waste products. The efficacy of MOL is primarily attributed to the synergistic activities of its constituent microbial populations, particularly the diverse bacterial consortia inherent to these solutions.

Bacterial consortia are pivotal in the composting process, driving the enhanced degradation of complex OWs. These consortia comprise various bacterial strains, each possessing specific enzymatic activities crucial for the breakdown of diverse organic compounds. Studies have demonstrated that such microbial consortia can markedly accelerate the composting of agricultural and kitchen waste, leading to more efficient waste management (Cao et al., 2022). Their efficacy stems from the production of key enzymes, including protease, lipase, amylase, and cellulose, which are instrumental in organic material decomposition (Oviedo-Ocaña et al., 2022). Therefore, the strategic application of bacterial consortia in composting offers a sustainable and environmentally sound approach to organic waste management, fostering nutrient recycling and promoting soil health.

The rumen, a specialized digestive organ in ruminants, hosts a highly efficient and diverse microbial ecosystem renowned for its exceptional ability to degrade complex plant polysaccharides. This unique environment makes rumen contents a compelling, yet underexplored, source of potent microbial bio-activators for various biotechnological applications, including composting. Despite this inherent potential, a significant research gap persists in comprehensively characterizing the specific multi-functional capabilities of rumen bacteria, particularly their combined cellulolytic, potassium-solubilizing, and phosphorus-solubilizing activities, for broader agricultural waste management applications. In this study, Bali cattle were specifically selected as the potential source for cellulolytic bacteria producers due to their well-documented adaptation to thrive on low-quality, high-fiber indigenous forages, which indicates a highly efficient and robust cellulolytic microbial community within their rumen system. In light of this background, this study aimed to explore and characterize the cellulolytic, potassium-solubilizing, and phosphorus-solubilizing potentials of bacteria isolated from the rumen of Bali cattle.

2. methodology

***Study Location and Duration***

This study was conducted at the Animal Husbandry Laboratory and Agronomy Laboratory, Faculty of Agriculture, University of Bengkulu, Indonesia, from November 2023 to June 2024.

***Ethical Approval***

This research was conducted according to the ethical standards and guidelines for the use of animals in scientific research. The study protocol was reviewed and approved by the Ethics Committee of the Research and Community Service Institute, University of Bengkulu, Indonesia, under approval number: 76/KER-LPPM/EC/2025.

***Sample Collection and Preparation***

Fresh rumen fluid (100 mL) was collected from Bali cattle immediately after slaughter at a local slaughterhouse in Bengkulu City. The samples were transported to the laboratory within two hours in sterile glass bottles placed in an ice-filled cooler box to maintain microbial viability. Upon arrival, the fluid was filtered to remove coarse particles and serially diluted using a sterile 0.85% NaCl solution for further microbial isolation.

***Sterilization Procedures and Media Preparation***

All media and glassware were sterilized using standard procedures. Media were autoclaved at 121°C and 15 psi. Glassware was dry-sterilized at 160°C for 2 hours, and metal tools were sterilized by ethanol flaming under a laminar airflow hood. Media used included CMC Agar, Pikovskaya Agar, Alexandrov Agar, Nitrogen-Free Bromothymol Blue (NFB) medium, Nutrient Agar (NA), Nutrient Broth (NB), and Salkowski reagent for IAA detection.

***Isolation and Preliminary Screening of Target Bacteria***

Bacteria were isolated from serially diluted rumen fluid using the spot inoculation method onto selective media for five target functions:

***Cellulolytic Activity Assay***

A single colony was inoculated onto a CMC medium and incubated at 37°C for 24–48 hours. Plates were flooded with 0.1% Congo red solution and left for 15 minutes, followed by washing with 1 M NaCl three times. A clear halo around colonies indicated cellulose degradation. The cellulolytic index (IS) was calculated as follows (adapted from Hidayatulloh *et al.,* 2022):

IS = (Clear zone diameter – Colony diameter) / Colony diameter

***Nitrogen-Fixation Ability***

Isolates were pre-cultured in NB for 24 hours, then 1 mL of suspension was inoculated into 9 mL of semi-solid NFB medium and incubated at 30°C for 10 days. A color change from green to blue indicated nitrogen fixation activity (Baldani et al., 2014). Isolates were pre-cultured in NB for 24 hours, then 1 mL of suspension was inoculated into 9 mL of semi-solid NFB medium and incubated at 30°C for 10 days. A color change from green to blue indicated nitrogen fixation activity (Baldani et al., 2014). For quantitative assessment, a standard curve was prepared using NH₄Cl at concentrations of 0.125, 0.25, 0.5, and 0.75 ppm. Each 50 mL NH₄Cl solution was mixed with 1 mL Nessler reagent and absorbance was measured to establish a linear regression (Juliasih et al., 2024).

***Phosphate Solubilization Assay***

Molten Pikovskaya agar (15 mL) was poured into sterile Petri dishes and solidified. A bacterial suspension (10 mL) from pure isolates was spot-inoculated and incubated at 37°C for 5 days. Transparent zones indicated phosphate solubilization due to organic acid production. Clear zones were measured every 48 hours up to 336 hours and the solubilization index was calculated following the method of Tarigan (2013).

***Potassium Solubilization Assay***

Alexandrov agar (15 mL) was poured into sterile Petri dishes and allowed to solidify. A bacterial suspension was spot-inoculated and plates were incubated at 37°C for 7 days. Clear zones indicated potassium solubilization via extracellular organic acids binding K⁺ ions (K₂HPO₄). The clear zone index was calculated as follows:

Clear zone index=

***Indole-3-Acetic Acid (IAA) Production:***

Isolates were cultured in NB supplemented with 0.1 g/L L-tryptophan at 28°C in the dark for 5 days. After incubation, the culture was centrifuged and 4 mL of the supernatant was mixed with 1 mL Salkowski reagent (12 g/L FeCl₃ in 429 mL/L H₂SO₄). After 24 hours in the dark at 28°C, the development of a pink color indicated IAA production. Absorbance was measured at 535 nm using a UV-VIS spectrophotometer.

3. results and discussion

***Bacterial Isolation and Characterization***

The total microbial load of Bali cattle rumen fluid revealed considerable variation across microbial groups, as shown in Table 1. The highest microbial density was observed in the control group with an average total plate count of 1.53 × 10⁹ CFU/mL. Among the functional groups, nitrogen-fixing bacteria (TPC N) showed the highest count with 3.0 × 10⁸ CFU/mL, followed by cellulolytic bacteria (TPC CMC) at 2.7 × 10⁸ CFU/mL. Potassium-solubilizing (TPC K), phosphate-solubilizing (TPC P), and IAA-producing bacteria (TPC IAA) exhibited lower counts, recorded at 6.7 × 10⁷, 4.3 × 10⁷, and 7.7 × 10⁶ CFU/mL respectively.

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| **Table 1** **shows total plate counts for various microbial groups in the rumen fluid.** | | |
|  | **Average total plate count/mL sample** | **Colony Forming Unit (CFU)/mL** |
|  | | |
| Control | 1.534.090.909 | 1.5 x 109 |
| Tpc P | 43.636.364 | 4,3 x 107 |
| Tpc IAA | 7.727.273 | 7,7 x 106 |
| Tpc N | 300.000.000 | 3 x 108 |
| Tpc CMC | 273.344.156 | 2,7 x 108 |
| Tpc K | 67.272.727 | 6,7 x 107 |
| ) | | |
| *TPC = Total Plate Count. Groupings based on functional assays: P = phosphate-solubilizing; IAA = indole-3-acetic acid-producing; N = nitrogen-fixing; CMC = cellulolytic; K = potassium-solubilizing.* | | |

***Bacterial Efficacy Assessment (Clear Zone Assay)***

A total of 44 bacterial isolates were screened for their functional abilities, including cellulolytic, phosphate-solubilizing, and potassium-solubilizing activities. The results of clear zone index (CZI) measurements are summarized in Table 2. Among the cellulolytic bacteria, isolates such as P-SP 10 (1.3) and IAA-SP 3 (1.7) showed the highest enzymatic activity as visualized by distinct halo zones on CMC medium (Figure 1A), followed by others with moderate indices such as N-SP 2 and P-SP 9. For phosphate-solubilizing bacteria, the highest indices were recorded in isolates P-SP 3 (2.4) and P-SP 5 (2.4), indicating strong phosphate-solubilizing potential (Figure 1B). Potassium-solubilizing activity was found in several isolates, with K-SP 3 (1.8) and K-SP 4 (1.8) exhibiting the highest index values. Some isolates, such as IAA-SP 2 and IAA-SP 3, demonstrated multiple functional potentials with moderate indices in all three tested activities (Figure 1C).

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| **Table 2** **Clear zone index of bacteria isolated from rumen fluid of Bali cattle** | | | |
| **Isolated code** | **Cellulolytic bacteria** | **Phosphate solubilizing bacteria** | **Potassium solubilizing bacteria** |
|  | | |  |
| Cmc-SP1 | - | - | 1.3 |
| Cmc-SP2 | - | - | 1.0 |
| Cmc-SP3 | - | - | 1.0 |
| K-SP 1 | - | 2.2 | 1.3 |
| K-SP 2 | - | 1.6 | 1.3 |
| K-SP 3 | - | 1.7 | 1.8 |
| K-SP 4 | - | 1.8 | - |
| K-SP 5 | 1.0 | 2.3 | 1.0 |
| K-SP 6 | 1.0 | 1.5 | 1.0 |
| K-SP 7 | - | 1.9 | 1.0 |
| P-SP 1 | 1.4 | 1.7 | 1.0 |
| P-SP 2 | 1.1 | 2.4 | 1.0 |
| P-SP 3 | 1.0 | 1.0 | 1.0 |
| P-SP 4 | 1.0 | 2.0 | 1.0 |
| P-SP 5 | 1.0 | 2.4 | - |
| P-SP 6 | 1.0 | 1.7 | - |
| P-SP 7 | 1.0 | 1.0 | 1.0 |
| P-SP 8 | 1.0 | 1.0 | 1.0 |
| P-SP 9 | 1.4 | 1.0 | 1.0 |
| P-SP 10 | 1.3 | 2.3 | 1.0 |
| N-SP 1 | 1.0 | 1.0 | 1.0 |
| N-SP 2 | - | 1.0 | 1.0 |
| N-SP 3 | 1.0 | 1.0 | 1.0 |
| N-SP 4 | 1.0 | 1.0 | 1.0 |
| N-SP 5 | - | - | 1.0 |
| N-SP 6 | - | - | - |
| N-SP 7 | - | - | - |
| N-SP 8 | - | - | - |
| N-SP 9 | - | - | - |
| N-SP 10 | - | - | - |
| N-SP 11 | - | - | - |
| N-SP 12 | - | - | - |
| N-SP 13 | - | - | - |
| N-SP 14 | - | - | - |
| N-SP 15 | - | - | - |
| N-SP 16 | - | - | - |
| N-SP 17 | - | - | - |
| IAA-SP 1 | - | - | 1.0 |
| IAA-SP 2 | 1.0 | 1.0 | 1.0 |
| IAA-SP 3 | 1.7 | 1.5 | 1.0 |
| IAA-SP 4 | 1.0 | 1.0 | - |
| ) | | |  |
| *Values represent the clear zone index calculated from the average of three replicates. “–” indicates*  *no visible halo zone formed. The cellulolytic activity was tested on CMC agar; phosphate-solubilizing activity on Pikovskaya medium; potassium-solubilizing activity on Alexandrov medium. All assays were conducted using the spot inoculation method at 37°C.* | | | |



**Figure 1 Clear zones formed on specific selective media indicating enzymatic activity of bacterial isolates.** *(A) cellulolytic bacterium on CMC medium showing cellulose degradation; (B) phosphate-solubilizing bacterium on Pikovskaya medium indicating inorganic phosphate solubilization; (C) potassium-solubilizing bacterium on Alexandrov medium showing the ability to solubilize insoluble potassium compounds.*

**DISCUSSION**

The present study demonstrates that the rumen fluid of Bali cattle contains a diverse and functionally significant bacterial community, particularly those capable of cellulolytic degradation, phosphate and potassium solubilization, nitrogen fixation, and IAA production. The initial isolation and purification process yielded 41 distinct bacterial colonies from serial dilutions of rumen fluid, highlighting the microbial diversity within this unique environment. Functional characterization revealed the presence of 3 cellulolytic, 7 potassium-solubilizing, 10 phosphate-solubilizing, 17 nitrogen-fixing, and 4 IAA-producing isolates. The total microbial load reached 1.53 × 10⁹ CFU/mL, with nitrogen-fixing bacteria being the most abundant (3.0 × 10⁸ CFU/mL), followed closely by cellulolytic bacteria (2.7 × 10⁸ CFU/mL). These results align with findings by Harun & Sali (2019), who emphasized that the availability of nitrogenous substrates and NH₃ concentration influences the growth of nitrogen-fixing bacteria in the rumen. Furthermore, the high number of cellulolytic bacterial is consistent with the findings of Safika *et al.* (2017), who noted that forage-based diets promote cellulolytic microbial populations. In comparison, Safika *et al.* (2017) reported a cellulolytic bacterial count of only 4.81 × 10⁻⁵ CFU/mL. The higher number observed in this study is attributed to the exclusive forage-based diet, without concentrate, which promotes the grow of cellulolytic bacteria. Since cattle do not possess the enzymes needed to break down cellulose into glucose, this role is carried out by microbes in the rumen. This finding is further supported by Firrincieli *et al.* (2024), who identified that the rumen environment is rich in fibrolytic bacteria capable of degrading plant cell wall polysaccharides. Rumen microbes also secrete ligninolytic enzymes such as lignin peroxidase and manganese peroxidase, which are essential for lignin decomposition. Additionally, the rumen is a primary environment for bacterial protein synthesis. The abundance of nitrogen-fixing bacteria, particularly those involved in nitrate and nitrite reduction, varies depending on the host species and diet composition. The composition and activity of microbial populations are shaped by dietary factors, including the roughage-to-concentrate ratio and nitrate presence in the feed. Key nitrate-reducing bacteria include *Selenomonas ruminantium*, *Veillonella parvula*, and *Wollinella* *succinogenes*. The cell density of *S. ruminantium* involved in nitrate reduction typically comprises 8–10% of the total *S. ruminantium* population, with approximately 10⁶ cells/mL. In contrast, total bacterial counts in goat rumen have been reported at approximately 10¹⁰ cells/mL (Yang *et al.*, 2016). According to Mirahsanti *et al.* (2022), the bacterial population in Bali cattle rumen fluid ranges from 32 × 10³ and 171 × 10³ CFU/mL, with an average of 74 × 10³ ± 47 × 10³ CFU/mL, highlighting the significant bacterial presence within the rumen ecosystem.

Clear zone assays provided functional validation of the bacterial isolates. The clear zone index (CZI) served as a proxy for enzymatic activity related to substrate degradation or nutrient solubilization. As noted by Hendricks (2015) and Tang *et al.* ( 2020), the appearance of halo zones serves a key indicator of microbial enzymatic function. In this study, 38 isolates exhibited clear zones indicating cellulose, phosphatase, or potassium-solubilizing activity. The highest cellulolytic index (1.7 mm) was recorded in isolate IAA-SP 3, with strong Congo red-stained halos. Similarly, phosphate-solubilizing isolates P-SP 2 and P-SP 5 had the highest indices (2.4 mm), while potassium-solubilizing isolates such as K-SP 3 and K-SP 4 also demonstrated significant activity (Herdiyantoro *et al.*, 2018). These enzymatic activities are underpinned by biochemical mechanisms. Phosphate-solubilizing bacteria produce organic acids, including citric, glutamic, lactic, and succinic that chelate Ca²⁺ and release bioavailable H₂PO₄⁻ (Annizah *et al.*, 2021; Sonia & Setiawati, 2022). Likewise, potassium-solubilizing bacteria produce acids that liberate K⁺ ions from mineral matrices, as described by Mohamed and Farag (2020). Such functional traits not only support nutrient cycling but also enhance compost quality and soil fertility (Annizah *et al.*, 2021; Yadav, 2022).

Several isolates showed multifunctional capacities. For example, IAA-SP 3 not only exhibited high cellulolytic activity but also solubilized phosphate and potassium. These traits suggest that bacterial isolates from the rumen can function synergistically in microbial consortia to decompose organic matter and release nutrients simultaneously. Etesami & Maheshwari (2018) and Arromrak *et al.* (2022) emphasized the ecological adaptability of such multifunctional microbes in diverse environments. Vessey (2003) also highlighted that genera like Bacillus and Pseudomonas are capable of enhancing plant growth by coordinating multiple nutrient-related functions. The high functional diversity of isolates in this study positions rumen-derived bacteria as effective candidates for compost bioactivation. Their use can reduce composting time, enhance decomposition, and increase the availability of essential nutrients such as nitrogen, phosphorus, and potassium. Dewilda *et al.* (2021) and Attwood *et al.* (2019) demonstrated that the application of rumen bacteria accelerates compost maturity and improves soil nutrient profiles. Zhan (2024) further noted the role of microbial communities in nutrient cycling and soil health enhancement through organic matter breakdown. This aligns with current global efforts toward sustainable agriculture and circular bioeconomy by utilizing agro-waste and microbial biodiversity for integrated nutrient management.

4. Conclusion

This study demonstrated that the rumen fluid of Bali cattle contains diverse bacteria with cellulolytic, potassium-solubilizing, and phosphorus-solubilizing capabilities. The isolation of multifunctional strains highlights the rumen as a valuable source of microbial bio-activators for applications in composting, soil fertility enhancement, and sustainable agriculture. The strong enzymatic activities observed suggest potential for industrial use, particularly in biomass degradation and biofertilizer development. Future studies should focus on molecular identification and functional validation to support their practical application in biotechnological processes.

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