



Rice straw decomposition as a source of nutrients and bacteria with potential antagonistic traits: Insights from two localities in Iloilo, Philippines

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Abstract

Rice straw is an abundant agricultural byproduct that contributes to soil fertility and supports diverse microbial communities. While organic decomposition is well-documented, the specific microbial dynamics and antagonistic potential inherent in the resulting decomposed rice straw remain under-explored. This study aimed to (1) evaluate the physicochemical characteristics of decomposed rice straw after three months of decomposition from two locations in Iloilo, Philippines (Zarraga and San Miguel), and (2) evaluate the antagonistic activity of bacterial isolates against *Escherichia coli* ATCC 25922 and *Staphylococcus aureus* ATCC 25923 using agar plug method. Physicochemical analysis showed higher phosphorus ($0.21 \pm 0.00\%$), potassium ($0.43 \pm 0.01\%$), and organic carbon ($14.52 \pm 0.88\%$) in Zarraga compared to San Miguel ($0.11 \pm 0.00\%$, $0.18 \pm 0.02\%$, and $3.97 \pm 0.21\%$, respectively; $p < 0.05$). Viable counts were also greater in Zarraga (11.69 ± 0.19 log CFU/g) than in San Miguel (9.67 ± 0.20 log CFU/g; $p < 0.05$). Among 30 isolates obtained, four inhibited *S. aureus*, with the strongest inhibition observed in San Miguel isolates SM9 (20.67 ± 1.15 mm) and SM2 (18.67 ± 1.53 mm). No isolates inhibited *E. coli*. These results may suggest that nutrient-rich conditions in Zarraga supported higher bacterial abundance, whereas the unique physicochemical conditions in San Miguel could favor isolates with stronger antagonistic activity. Overall, the findings indicate that decomposed rice straw provides both nutrients and microbial communities with potential antagonistic traits. Further molecular and functional characterization is needed to identify these isolates and evaluate their relevance for agricultural and ecological potential.

Keywords: Rice straw decomposition, Bacterial antagonism, Physicochemical properties

1. Introduction

Rice straw, a residual byproduct left after rice harvesting, is a valuable substrate for decomposition and holds promise as an alternative organic fertilizer. Studies have shown that rice straw contains substantial levels of macronutrients (nitrogen, phosphorus, potassium) [1] and essential micronutrients (zinc, manganese, copper, iron), which together can significantly enhance soil fertility and support crop growth [2]. Yet the release and bioavailability of these nutrients depend critically on microbial activity [3]. Microorganisms drive the decomposition of organic residues, transforming complex organic matter into simpler, inorganic forms that plants can readily absorb [4]. Understanding this decomposition process is vital for optimizing the use of rice straw as a sustainable nutrient source in agricultural systems.

Microorganisms present in organic fertilizers not only contribute to nutrient cycling but also produce bioactive metabolites that can suppress the proliferation of harmful pathogens [5, 6]. This antagonistic activity enhances the disease-suppressive properties of compost-amended soils and strengthens plant resilience. For example, Reddy et al. [7] reported the isolation of bacterial strains from municipal solid waste compost that showed antagonistic activity against various pathogenic microorganisms such as *Salmonella* sp., *Staphylococcus aureus*, and *Klebsiella pneumoniae*. In a related study, Low et al. [8] investigated the diversity of actinomycetes recovered from five different manure composts and evaluated their antibacterial potential. Their results revealed a broad spectrum of antagonistic activity: seven isolates inhibited the growth of *Staphylococcus aureus*, five isolates suppressed *Bacillus subtilis*, four isolates impeded *Escherichia coli*, and one isolate was active against *Pseudomonas aeruginosa*. Similarly, Chen et al. [9] highlighted the agricultural benefits of this antagonism, noting that bacteria from rice straw could inhibit *Rhizoctonia cerealis*. These findings highlight the multifaceted role of compost microorganisms, not only in facilitating organic matter decomposition and nutrient release but also in enhancing the biological control potential of organic fertilizers by limiting pathogenic microbes.

Building on this background, the present study hypothesizes that during the decomposition of rice straw, microorganisms generate metabolites that not only facilitate nutrient release but also enhance antagonistic interactions within the microbial community. While soil microorganisms have long been studied for their diverse metabolites, many of which have demonstrated applications in agriculture, medicine [10], and the pharmaceutical industry [11]. Less attention has been directed toward microbial dynamics and antagonistic activity within decomposed organic fertilizers themselves. Addressing this gap, the current research aims to evaluate the antagonistic potential of bacterial isolates obtained from decomposed rice straw against *Escherichia coli* and *Staphylococcus aureus*. Furthermore, an analysis of the physicochemical characteristics of decomposed rice straw obtained from two representative agricultural areas in Iloilo, Philippines was carried out. Together, these assessments seek to advance our understanding of rice straw as a dual-function input which serves both as a nutrient source and as a reservoir of beneficial microorganisms with antagonistic properties.

2. Materials and methods

2.1 Study site

Rice straw samples were collected from two agricultural sites in Iloilo, Philippines: Barangay Dawis Norte in Zarraga (Orange circle) and Barangay Consolacion in San Miguel (Red circle) (Figure 1). The first sampling site, Dawis Norte (10°52'02.0"N, 122°37'05.2"E), covers an estimated area of 92.03 hectares. Rice cultivation is the predominant livelihood in this community, supplemented by small-scale poultry and livestock production, including chickens, pigs, and goats. The second sampling site, Barangay Consolacion (10°48'06.5"N, 122°28'26.4"E), has an area of approximately 3,197 hectares and an elevation of about 40.3 meters (132.2 feet) above mean sea level. Similar to Dawis Norte, rice farming is the primary source of livelihood, with residents also engaged in raising poultry and large livestock such as carabaos, cows, and goats.

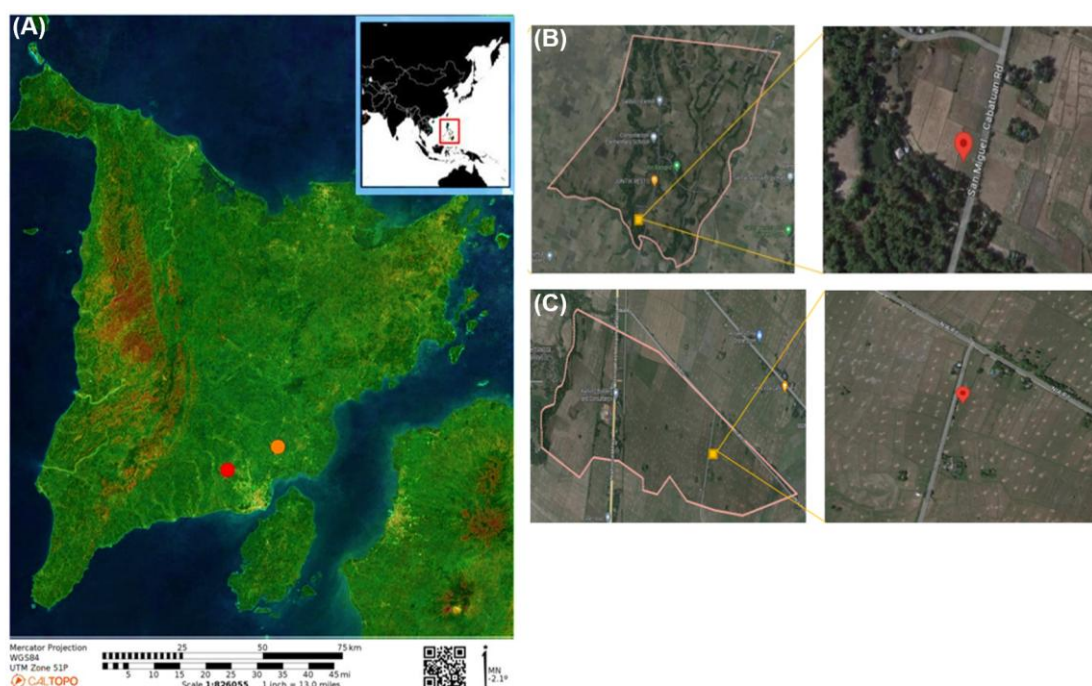


Figure 1 Study Site. (a) The map of Panay highlighting Barangay Dawis Norte in Zarraga (Orange circle) and Barangay Consolacion in San Miguel (Red circle); (b) Map of Barangay Consolacion in San Miguel highlighting the actual collection site (Red mark); and (c) Map of Barangay Dawis Norte in Zarraga highlighting the actual collection site (Red mark).

2.2 Sample collection and preparation

Raw rice straw samples were collected from two sampling sites on March 17, 2022. Collection was carried out in the middle of the rice fields, where raw rice straw residues had been left after harvesting. The gathered rice straw was placed in clean, sterile containers with plastic liner for heat retention and water control and stored under conditions that minimized external contamination. Following collection, the samples were broken down into smaller pieces using scissors and placed in a designated area where they remained undisturbed throughout the decomposition process. The sample was exposed only to natural environmental factors such as sunlight and rainfall, ensuring that decomposition occurred under ambient, field-relevant conditions (exactly as it would on a local farm). The rate of decomposition was monitored for three months until the rice straw was fully decomposed as evaluated through visual cues (rice straw structure is completely gone, soil-like consistency, chocolate-brown color, etc.), providing a basis for subsequent microbial and physicochemical analyses (Figure 2).

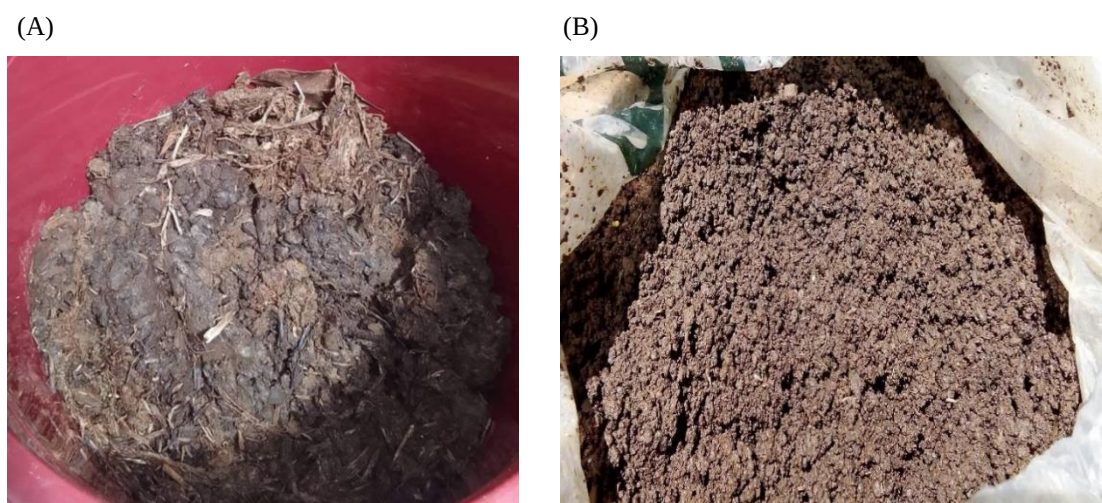


Figure 2 Example of raw rice straw samples (A) before and (B) after three months of decomposition process.

2.3 Physicochemical Properties

Approximately 1 kg of decomposed rice straw samples were submitted to the Regional Soil Laboratory VI for physicochemical analysis, including the determination of potassium (K), phosphorus (P), organic matter (OM), moisture content, and pH [12].

2.4 Bacterial assessment

Following the manufacturer's protocol, Nutrient Agar (Scharlau) was prepared and sterilized in an autoclave at 121 °C and 15 psi for 15 minutes. The diluent was formulated based on the U.S. Food and Drug Administration [13] guidelines by dissolving 1 g of peptone in 1 L of distilled water and sterilizing it under the same conditions. The final pH was adjusted to 7.0 ± 0.2 . Serial sample dilutions were conducted using a modified procedure of Shen et al. [14]. In this process, 25 g of decomposed rice straw was added to 225 ml of 0.1% peptone solution, and a 1 ml aliquot from the homogenized suspension was transferred into 9 ml of sterile diluent to achieve a 10^{-1} dilution. Successive ten-fold serial dilutions were prepared up to 10^{-10} . For bacterial isolation, quadrant streaking was performed to obtain distinct colonies. Subculturing of representative colonies was carried out repeatedly until pure cultures were obtained. Colonies exhibiting distinctive pigmentation were used as the screening criterion and selected for further isolation. Pure cultures were confirmed by Gram staining, and observations were made under a light microscope using oil immersion [15]. This allowed the confirmation of morphological characteristics and the verification of culture purity prior to subsequent analyses.

2.5 Antagonistic activity and colony morphology

The isolated bacterial colonies were evaluated for their antagonistic potential against two standard test organisms: *Staphylococcus aureus* ATCC 25923, representing Gram-positive bacteria, and *Escherichia coli* ATCC 25922, representing Gram-negative bacteria. Antagonistic activity was assessed using the agar plug method, as described by Tupas et al. [15]. Briefly, agar plugs containing the bacterial isolates were transferred onto nutrient agar plates previously inoculated with the indicator bacteria. The plates were incubated at 37 °C for 24 hours. Following incubation, zones of inhibition surrounding the agar plugs were measured in millimeters using a metric ruler to determine the extent of growth suppression. To ensure reproducibility, each assay was conducted in two independent trials with duplicate plating, thereby minimizing the likelihood that inhibition occurred by chance. Bacterial isolates exhibiting inhibitory activity were further categorized based on (1) Gram-stain reaction and (2) colony morphology observed on agar plates. Morphological traits recorded included colony margin, texture, color, elevation, and shape [16, 17].

2.6 Statistical Analysis

To assess the antagonistic effect against *S. aureus* ATCC 25923, data were subjected to analysis of variance (ANOVA) with Tukey's post hoc test for identifying significant differences. The independent samples t-test was applied to evaluate variations in nutrient composition and CFU counts, using IBM SPSS Statistics version 20 for data processing.

3. Results

3.1 Physicochemical parameters

Figure 3 presents the results of physicochemical analysis of decomposed rice straw collected from Zarraga and San Miguel. The mean phosphorus content in Zarraga ($0.21\% \pm 0.00$) was nearly twice that of San Miguel ($0.11\% \pm 0.00$). Similarly, potassium content was higher in Zarraga ($0.43\% \pm 0.01$) compared with San Miguel ($0.18\% \pm 0.02$) (Figure 3a). Statistical evaluation revealed significant differences ($p < 0.05$) in both nutrients between the two sites, indicating that rice straw decomposed in Zarraga released greater amounts of phosphorus and potassium than that from San Miguel. In contrast, organic carbon content (Figure 3d) was markedly higher in Zarraga ($14.52\% \pm 0.88$) compared with San Miguel ($3.97\% \pm 0.21$), with a notable difference ($p < 0.05$). As shown in Figure 3b, the pH levels of decomposed rice straw also differed significantly between the two sites ($p < 0.05$). San Miguel samples recorded a mean pH of 7.16 ± 0.04 , while Zarraga samples were near-neutral with a mean pH of 6.94 ± 0.06 . Moisture content analysis revealed that decomposed rice straw from San Miguel retained more water ($40.52\% \pm 0.19$) than that from Zarraga ($34.55\% \pm 0.88$), with the difference again being statistically significant ($p < 0.05$) (Figure 3c). This indicates that rice straw decomposed in San Miguel may have a greater capacity to hold water, which could influence microbial activity and decomposition dynamics.

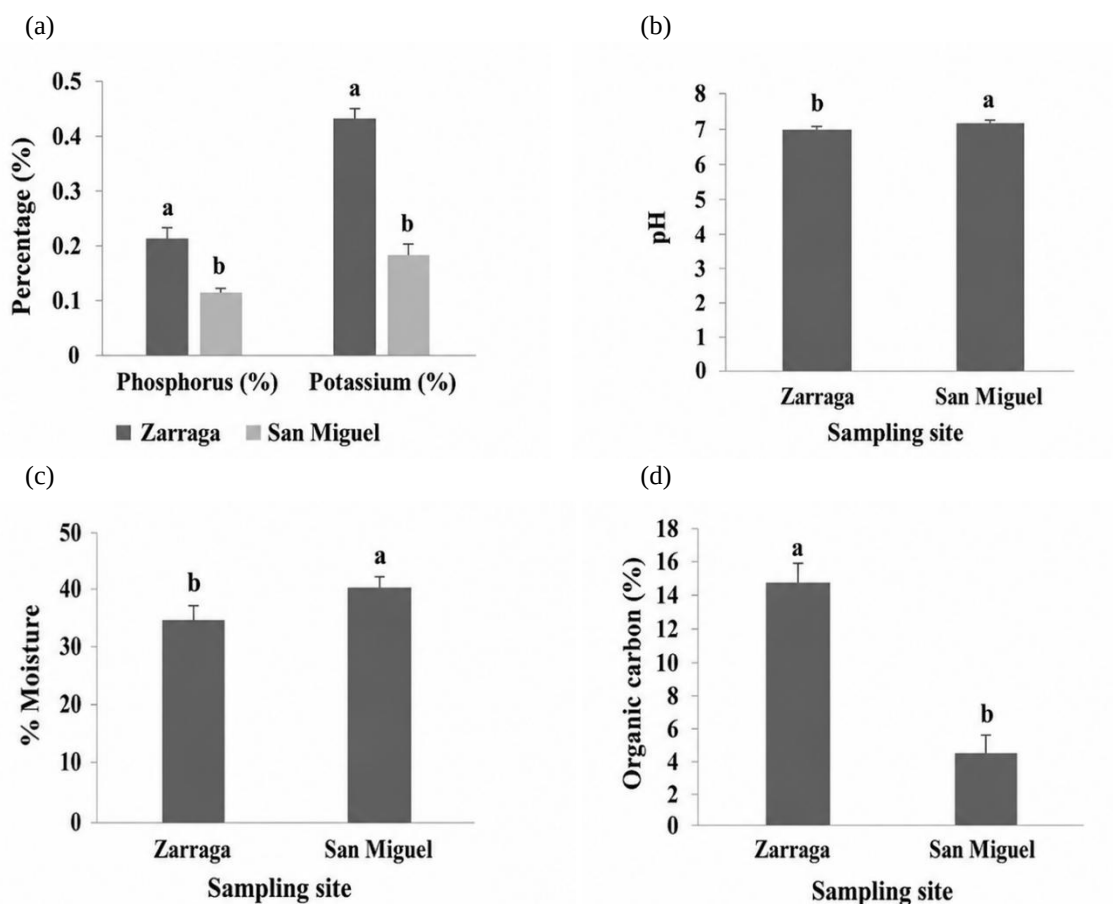


Figure 3 Physicochemical properties of decomposed rice straw between the two sites. (a) Phosphorus and Potassium; (b) pH; (c) Moisture; and (d) Organic carbon. Data are presented as mean $\pm$ standard deviation (SD) of three replicates. For each nutrient, means with identical superscript letters are not significantly different ($p > 0.05$).

3.2 Colony Forming Unit

The mean bacterial population, expressed as log CFU/g, was calculated from the actual CFU counts obtained from decomposed rice straw samples (Figure 4). Results indicated that rice straw from Zarraga harbored significantly higher bacterial populations (11.69 ± 0.19 log CFU/g) compared with those from San Miguel (9.67 ± 0.20 log CFU/g). Statistical analysis confirmed a significant difference between the two localities ($p < 0.05$). These findings suggest that rice straw decomposition in Zarraga supports greater microbial proliferation than in San Miguel.

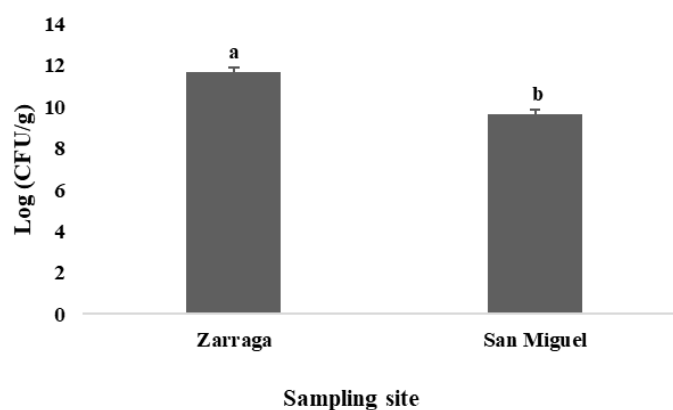


Figure 4 Bacterial population expressed as log CFU/g. Data are presented as mean $\pm$ standard deviation (SD) of three replicates. Colony forming unit values with identical superscripts show no significant variation ($p > 0.05$).

3.3 Antagonistic activities of isolates

Based on colonies exhibiting distinctive pigmentation, a total of 30 bacterial isolates obtained from decomposed rice straw were screened for their ability to inhibit the growth of (A) *Staphylococcus aureus* ATCC 25923 and (B) *Escherichia coli* ATCC 25922 (Table 1). Antagonistic activity was determined based on the presence of clear inhibition zones surrounding the colonies of the test isolates. In Table 1, isolates exhibiting positive antagonism are highlighted in red text for clarity. Out of the 30 isolates tested, only four demonstrated inhibitory activity against *S. aureus* ATCC 25923. These were identified as isolates Z9 and Z13 from Zarraga, and SM2 and SM9 from San Miguel. None of the isolates exhibited detectable antagonism against *E. coli* ATCC 25922.

Table 1 Presence and absence of antagonistic activity of isolates against (A) *E. coli* ATCC 25922 and (B) *S. aureus* ATCC 25923.

Bacterial Isolate No.	Zarraga		Bacterial Isolate No.	San Miguel	
	A	B		A	B
1	-	-	1	-	-
2	-	-	2	+	-
3	-	-	3	-	-
4	-	-	4	-	-
5	-	-	5	-	-
6	-	-	6	-	-
7	-	-	7	-	-
8	-	-	8	-	-
9	+	-	9	+	-
10	-	-	10	-	-
11	-	-	11	-	-
12	-	-	12	-	-
13	+	-	13	-	-
14	-	-	14	-	-
15	-	-	15	-	-

Symbol: (+) Presence; (-) Absence

Among the four isolates that exhibited inhibitory effects, the strongest activity was observed in isolate SM9 from San Miguel, which produced the largest zone of inhibition (20.67 ± 1.15 mm). This was followed by isolate SM2, also from San Miguel, with a mean inhibition zone of 18.67 ± 1.53 mm. In comparison, isolate Z9 from Zarraga produced an inhibition zone of 16.67 ± 1.15 mm, while isolate Z13 from the same site exhibited the lowest activity, with a zone of 13.33 ± 1.15 mm (Figure 5). Statistical analysis confirmed a highly significant difference in the inhibition zones among the four isolates ($p < 0.05$).

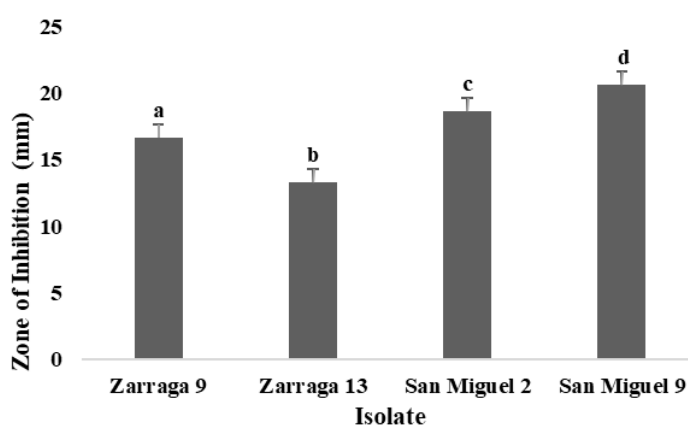


Figure 5 Antagonistic activity of selected bacterial isolates against *S. aureus* ATCC 25923. * Data are presented as mean $\pm$ standard deviation (SD) of three replicates. Zone of inhibition values with identical superscripts show no significant variation ($p > 0.05$).

The isolates exhibiting antagonistic potential were assessed for colony morphological characteristics following the criteria outlined by Breakwell et al. [17]. The descriptive parameters included colony margin, color, elevation, texture, shape, and Gram stain reaction. It is important to note, however, that colony morphology alone is not a definitive criterion for species identification, as different bacterial species or strains may display overlapping morphological features. Nevertheless, such observations provide useful preliminary insights into bacterial diversity and phenotypic variation. Among the antagonistic isolates, distinct morphological differences were

observed (Table 2). Isolates Zarraga 13 and San Miguel 9 displayed entire margins, whereas Zarraga 9 exhibited a filamentous margin and San Miguel 2 showed a curled margin. All isolates formed circular colonies in shape, although their elevations varied: Zarraga 13 was raised, while Zarraga 9, San Miguel 2, and San Miguel 9 were flat. Pigmentation also differed across isolates. Both San Miguel isolates (SM2 and SM9) produced a yellow pigment, while Zarraga 9 was white, and Zarraga 13 produced a distinctive brown coloration. Colony texture further distinguished the isolates: Zarraga 9 and San Miguel 2 exhibited wrinkly/dry textures, Zarraga 13 was mucoid/slimy, and San Miguel 9 presented a matte surface. Gram staining confirmed that all four isolates were Gram-positive bacteria. Morphological assessment under the microscope revealed that the majority were bacilli, with the exception of Zarraga 9, which displayed a coccus morphology.

Table 2 Morphological characteristics of bacterial isolates with antagonistic activity against *S. aureus* ATCC 25923

Isolate	Margin	Color	Elevation	Texture	Shape	Gram Stain
Z9	Filamentous	White	Flat	Wrinkly/ Dry	Round	Gram Positive Coccus
Z13	Entire	Brown	Raised	Mucoid/ Slimy	Round	Gram Positive Bacillus
SM2	Curled	Yellow	Flat	Wrinkly/ Dry	Round	Gram Positive Bacillus
SM9	Entire	Yellow	Flat	Matte	Round	Gram Positive Bacillus

Abbreviations: (Z) Zarraga; (SM) San Miguel.

4. Discussion

4.1 Physicochemical Properties

The study demonstrated clear site-specific differences in the physicochemical parameters of decomposed rice straw. Zarraga samples contained higher phosphorus, potassium, and organic carbon, while San Miguel samples had higher pH and moisture compared to Zarraga. These variations can be explained partly by fertilizer practices and livestock contributions. Long-term P and K fertilization has been shown to enrich straw nutrient content and influence residue chemistry [18]. The higher organic carbon in Zarraga suggests slower mineralization, whereas elevated moisture in San Miguel likely accelerated decomposition and carbon turnover, consistent with findings that excess water availability promotes carbon loss during composting [19]. Moreover, pH and moisture often covary [20], aligning with the neutral to slightly alkaline conditions observed in San Miguel.

Overall, these findings illustrate how management practices and environmental factors influence nutrient composition during decomposition, with Zarraga favoring nutrient retention and San Miguel favoring faster mineralization.

4.2 Viable Cells

Bacterial counts were significantly higher in Zarraga, correlating with its greater nutrient content. Nutrient-rich conditions provide energy sources that sustain microbial proliferation during decomposition [21]. Similar studies confirm that microbial abundance shifts over time, with copiotrophic bacteria dominating early decomposition stages and oligotrophs persisting later [22, 23]. The consistently high viable counts from Zarraga suggest that nutrient-enriched residues may support more robust microbial communities, while San Miguel conditions appeared less favorable for microbial proliferation.

4.3 Antagonistic Activity

Of the 30 isolates recovered, only four inhibited *Staphylococcus aureus*, with no activity against *Escherichia coli*. This selective inhibition reflects structural differences between Gram-positive and Gram-negative bacteria, as Gram-positive cell walls are generally more susceptible to bacteriocins and lipopeptides [8]. Interestingly, San Miguel isolates (SM2, SM9) exhibited stronger inhibition despite lower bacterial abundance. This suggests that the environmental condition in San Miguel, may have imposed selective pressures that promoted antimicrobial metabolite production as a survival strategy [24, 25].

Morphological traits suggested these isolates may belong to *Bacillus* or Actinomycetes, both of which are common in decomposing residues and known for producing secondary metabolites with antagonistic properties [26, 27]. *Bacillus*, in particular, has been reported as an efficient antagonist against Gram-positive bacteria and is frequently isolated from composts and crop residues [28, 29]. While preliminary morphological characterization provides useful insights, molecular identification and metabolite profiling are required to confirm taxonomic placement and functional potential.

4.4 Relationship of physicochemical parameters, bacterial growth, and antagonistic activity

The contrasting outcomes between Zarraga and San Miguel highlight an ecological trade-off. In Zarraga, nutrient-rich conditions favored microbial proliferation but not strong antagonistic traits. In San Miguel, its physicochemical conditions possibly supported fewer bacteria but enhanced antagonistic activity. This is consistent with ecological models where resource abundance drives population size, whereas stressful or unique environments promote specialization and metabolite production [30, 31]. Thus, rice straw decomposition represents both a nutrient recycling process and a context for microbial interactions that shape community function. Understanding these dynamics is critical for designing management practices that maximize both soil fertility and the potential disease-suppressive effects of residue amendments [32, 33].

5. Conclusion

This study showed that decomposed rice straw supports nutrient recycling and harbors bacteria with antagonistic activity against *S. aureus*. Zarraga samples were nutrient-rich and supported higher bacterial counts, while San Miguel samples, despite lower microbial abundance, yielded isolates with stronger antagonistic activity against *S. aureus*. These results suggest that physicochemical conditions not only shape microbial density but also influence functional traits such as antimicrobial metabolite production. Present results indicate that rice straw decomposition may play dual ecological roles: (1) enhancing soil fertility through nutrient recycling, and (2) providing a reservoir of microorganisms with potential antagonistic properties. Further molecular and functional identification and characterization are required to determine their potential role in sustainable crop protection

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7. Author Contributions

Tamondez, R., Aghayar, Y., Sinco, A., Tena, M.: Conceptualization, Methodology, Writing – original draft, Investigation, Formal analysis, Data curation; Billonid, J., Yerro, E., Yerro, P., Arabaca, M.L., Caipang C.: Conceptualization, Writing – review & editing, Supervision. All authors reviewed the manuscript and approved the final version.

8. Conflicts of Interest

The authors declare no conflict of interest.

9. References

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