FUDMA Journal of Sciences (FJS)
ISSN online: 2616-1370
ISSN print: 2645 - 2944
Vol. 10 No. 10, May, 2026, pp 137 – 143
DOI: https://doi.org/10.33003/fjs-2026-1010-5423

Earthworm Casts as Selective Microhabitats Enriching Antagonistic Bacteria with in Vitro Inhibitory Potential against Enteric Bacterial Pathogens

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ABSTRACT
Earthworm casts as biologically active microhabitats significantly influence soil microbial compositions and activities. Analysis was conducted to evaluate the diversity and antagonistic potential of bacterial isolates in the casts of earthworm and adjacent soil against some selected enteric bacterial pathogens. A total of 30 bacterial isolates, comprising 18 isolates from earthworm casts and 12 isolates from the soil samples, were obtained. Morphological and biochemical characterization revealed the presence of dominant genera, including: *Bacillus* spp., *Streptomyces* spp., and *Pseudomonas* spp. Antagonistic activity was assayed using the agar well diffusion method against *Escherichia coli*, *Salmonella* spp., and *Klebsiella* spp. Among the total isolates obtained, 14 (46.7%) exhibited antagonistic activity, with a higher proportion originating from earthworm casts, and 10 isolates with 71.4% compared to soil, which had 4 isolates; 28.6%. The zones of inhibition ranged from 8 mm to 22 mm across all test organisms. *Bacillus spp.* demonstrated the highest inhibitory activity with a mean zone of 18 mm, followed by *Streptomyces* spp. (15 mm), while *Pseudomonas* spp. showed a comparatively lower activity. *Escherichia coli* was the most susceptible test pathogen, exhibiting a maximum inhibition zone of 22 mm, whereas *Klebsiella* spp. showed the least susceptibility with a minimum inhibition reading of 10 mm. Negative control experiments showed no zones of inhibition, confirming that antimicrobial effects were exclusively due to metabolites produced by the assayed bacterial isolates. The outcome of the finding suggests that earthworm cast may serve as a selective microenvironment that enriches bacterial populations with significant antagonistic potentials.

Keywords: Antibacterial, Antibiosis, Biocontrol and Vermicompost

INTRODUCTION

Earthworms are abundant soil terrestrial organisms that egest soil, minerals, plant matter, nutrient particles, microorganisms, and other organic particles in the soil systems (Blouin *et al.*, 2013 & Lavelle *et al.*, 1995). The component of their guts is decomposed for transformation, mixed with mucus, and other parts of their fecal materials to stabilize the walls of their burrows. They deposit the leftovers of this mixture on the surface of soils as casts (Rogasik *et al.*, 2014). Earthworm casts deposited on the surface of soils are often rich in nutrients and organic substances that are usually more fertile even as soil amendments, when compared to those of bulk soils in the environment (Van Groningen *et al.*, 2019; Sharma *et al.*, 2025; Medina-Sauza *et al.*, 2019). Furthermore, the activity of earthworms and their casts released from the gut passage helps to improve soil structures in places where they make influence. Earthworm activities in soil systems help in macropore formation, while their cast contents may often help to promote soil aggregation as well as introduce selected microbial species to the soil (Zhang and Schrader, 1993; Marashi and Scullion, 2003; Khyade, 2018).

Enteric bacterial pathogens exposed to soils may find their way into the guts of earthworms, but their survival may usually depend on the microbial interactions that often exist within the guts and cast before their release to soil systems. In terms of prevalence, some bacterial species may be reduced or completely eradicated when they pass through the guts of earthworms, as demonstrated by ingested cells of *Escherichia coli* (Pedersen and Hendriksen, 1993; Thimm *et al.*, 2001; Aira *et al.*, 2024; Tiwari *et al.*, 2015). Changes to microbial community structures during and after the passage of microbial populations have been demonstrated in works by Cai *et al.*, 2002; Nechitaylo *et al.*, 2010; Gong *et al.*, 2018; Buivydaite *et al.*, 2023; and Sofo *et al.*, 2023; Gómez-Brandón *et al.*, 2011; Yang *et al.*, 2024. Earthworms are recognized to influence and modify soil microbial communities by the passage of materials through their guts, exposure to transient microorganisms, and by what is released as products in their cast (Blouin *et al.*, 2013).

Antagonistic Bacterial species like *Bacillus* spp., *Pseudomonas* spp., and *Streptomyces* spp. live not only in soils, but also in the guts and cast of earthworms, where they produce secondary metabolites that may defend against certain pathogens. The burrowing and casting activities of earthworms help to create the drilosphere zone of intense microbial activity, which makes them crucial as ecosystem engineers (Sharma *et al.*, 2025).

Although the activities of earthworms mostly create drilosphere zones in soils, their guts have been well known to act as a selective and optimal microbial growing system, like bioreactors, which provide anoxic systems of intestinal mucus that may potentially trigger what is known as a priming effect on some dormant soil microorganisms (Sharma *et al.*, 2025). According to Gómez-Brandón *et al.*, (2011), during gut transit of most microorganisms, the microbiome colonising such networks often undergoes a bottleneck effect as the total microbial biomass may decrease, with mostly copiotrophic taxa becoming selectively enriched.

The outcome of earthworm cast is well known as microbial hotspots with higher significant bacterial populations and enzymatic activities than those that have been experimented in bulk soils (Yang *et al.*, 2024; Khyade, 2018; Sharma *et al.*, 2025).

Extensive works by Kumar *et al.*, (2012) and Devi and Serfoji (2018) establish that environments that are influenced by earthworms, such as casts and the guts of earthworms, may foster antagonistic microbial populations that help to suppress diseases in soils. Reports on vermicomposting are known to

provide evidence of lowering bacterial pathogens of humans, like *Escherichia coli* and *Salmonella* spp., for up to 95 to 100 percent reductions in bacterial load. This is influenced by the impact of gut digestion and other activities of antagonistic flora released in enriched earthworm casts (Tiwari *et al.,* 2015; Aira *et al*., 2024). However, it is important to note that the role of naturally ageing earthworm casts may require further exploration, particularly as relating to the acceptance of the in vitro validation against tested human enteric pathogens.

MATERIALS AND METHODS

Media used for the Isolation and Culturing of Bacterial species

Nutrient agar was used as a general-purpose medium for the isolation, culturing, and enumeration of total heterotrophic bacterial species, while Starch casein agar (subjected to incubation of cultures at 28°C for 7–14 days) was used for the selective recovery of Actinomycetes, *Streptomyces* spp., from the samples (Kumar *et al.,* 2012). Other selective media used were *Pseudomonas* Agar, *Salmonella*/*Shigella* Agar, and MacConkey Agar. All agar used except Starch casein agar was incubated at 37°C for 24–48 hours. Distinct colonies from all cultured agar were sub-cultured onto slants maintained at 4°C in order to get pure cultures using aseptic techniques and methods of streak plating for ensuring purity and further testing.

Earthworm Cast, Sample Collection, and Preparation

12 Adult *Lumbricus terrestris* earthworms were dug out from an agricultural soil and taken to the laboratory for processing. They were washed 3 times using sterile distilled water in order to remove the debris and soil particles that adhered to them. The earthworm specimens were divided into 3 groups and put inside 4 sterile plastic Petri dishes with moistened filter papers, lined inside them, and left at about 20°C for between 24 and 48 hours, in order to allow the earthworms to clear the contents of their guts. A sterilized spatula was then used to collect the freshly deposited casts from the filter papers for isolation purposes. 1 gram of the collected cast materials was suspended in a 99 ml quantity of sterile saline solution of 0.9% NaCl and then transferred into a rotatory shaker that ran for up to an hour in order to homogenize the samples. This was done according to the methods by Vishnu (2021)

Sample Collection and Isolation

Earthworm casts and surrounding bulk soil were collected to serve as sources for microbial isolation. Adult earthworm specimens were washed and placed in sterile Petri dishes to allow for gut clearance (Sharma *et al.,* 2025). Bacterial isolates were recovered using the serial dilution agar plating technique (Tiwari *et al*., 2015). Initial homogenates were then diluted and inoculated onto Nutrient Agar (NA) and Actinomycete Isolation Agar (AIA) (Kumar *et al.,* 2012; Vishnu, 2021). A total of 40 isolates (soil and casts) were obtained for evaluation.

Collection of Surrounding Soil (As Control Sample)

For the purpose of comparative analysis, one gram of surrounding soil was aseptically collected as control samples from around the same depths and regions equivalent to those where earthworms were collected for their cast. All soil samples that were collected were subjected to homogenization with the same methods as those carried out for earthworm casts (Vishnu, 2021). This soil sample is the control sample that was needed to evaluate the potential of microbial enrichments in the cast of earthworms (Yang *et al.,* 2024).

Isolation of Bacterial Species from Earthworm Casts and Surrounding Soils

Bacterial isolation was carried out by using methods of Serial dilution and Agar plating technique (Tiwari *et al.,* 2015). Contents of both soil and cast homogenates were serially diluted to concentrations of 10^3. Aliquots of 0.1 ml of the dilution were aseptically inoculated into selective and non-selective media in order to recover a diverse population of bacterial species and hence minimize the dominance of some fast-growing microorganisms.

Morphological and Biochemical Characterization

Morphology and Gram staining of the isolates: Isolates were examined through their morphological appearance on the plates, such as shapes, margins, colour, and elevation. Gram staining was used to determine their cell shapes, staining colours, as well as their KOH test in 3% solution to confirm their Gram reactions. Motility testing was also determined using the hanging drop technique. These tests were done following the descriptions in Bergey's Manual of Systematic Bacteriology, 9th edition.

Biochemical Identification of the Isolates

Sub-cultured isolates were subjected to a series of standard tests following guidelines given by Bergey's Manual of Systematic Bacteriology, 9th edition. These tests included: IMViC Tests such as Indole Production, Voges-Proskauer (VP) test, Methyl Red (MR) Test, and Citrate utilization for differentiating coliforms from other enteric genera (Vishnu, 2021). Test for Enzymatic activities, involving: Oxidase test and Catalase test (using 3%) as well as Starch hydrolysis on starch agar, flooded with iodine for amylase determination, and Triple sugar (TSI) Iron Agar test, which were done to determine the ability of the isolates to ferment lactose, glucose, and also to produce hydrogen sulfide (Evangeline, 2023).

Target Pathogens and Culture Maintenance

Pathogens of enteric human and bacterial pathogens, which were used as indicator bacterial test strains for antagonism studies, included: *Escherichia coli*, *Salmonella* spp., and *Staphylococcus aureus* (Aira *et al.,* 2024). These pathogens were aseptically cultured and maintained on Nutrient Agar slants and then sub-cultured into nutrient broths for 24 hours before further antagonism assays. Each pathogen was cultured in nutrient broth for about 18–24 hours and then adjusted to a 0.5 McFarland standard of ~1×10^8 CFU/mL, using sterile normal saline. This standardization ensured that there was a uniform inoculum diffusion density across all the assays.

Testing for Antagonistic Activity in Soil and Earthworm Cast

Primary and secondary Antagonism, testing methods were both used to evaluate the inhibitory potential of earthworm cast-derived isolates and soil isolates (Turin *et al.,* 2025).

Cross Streak Primary Testing Method for Antagonistic activity

The cross-streak method was carried out as a primary screening method, using each isolate to streak a single central line onto an already prepared nutrient agar and starch casein agar plates, before incubating them between 28–30°C for about 48–72 hours to give room for the production of metabolites. Subsequently, the experimental test pathogens

were streaked perpendicularly onto the colonial growth of the isolates and then incubated at around 37°C for up to 24 hours. Evidence of antagonism activity was further assessed by the Prescence and extent of inhibition of the growth of the pathogens being tested.

Secondary Screening Method using Agar Well Diffusion Tests

Mueller-Hinton agar plates were prepared and seeded with the standardized inoculum preparation of the pathogens at 0.5 McFarland. Round wells of about 6 mm in diameter were aseptically drilled using a cork borer onto all the plates. The contents of 0.1ml of all the centrifuged cell-free culture supernatant, which were obtained after 24 – 48 hours of broth culturing, were then transferred into each well for testing (Evangeline, 2023).

Measurement of Zones of Inhibition (ZOI):

All the plates tested for antagonism studies were incubated at 37°C for 24 hours and then checked for evidence of inhibition. Cultured plates that had signs of inhibition were measured using a ruler in millimetre units and expressed as zones of inhibition (ZOI). To ensure the reproducibility of results, all assays were performed in triplicate.

Negative Control for Antagonism Testing

Sterile nutrient agar broth was used to test for both earthworm cast and soil samples to confirm the absence of inhibition that may be inherent, while Ampicillin was used as a standard antibiotic as a positive control for the validation of the assay sensitivity.

Statistical Analysis of Data

Experimental isolates were tested in replicates, and their mean inhibition zones per isolate were used for statistical analysis. One-way Anova was used to assess the variation in inhibition across sampled sources of the different groups of pathogens (Turin *et al.*, 2025). The student's T-test, assuming unequal variance, was used to compare the overall mean of the soil and cast samples. It was done for the Agar well diffusion test, using the formula =T.TEST (A2:A21, B2:B21, 2, 3) Where: 2 = two-tailed and 3 = unequal variance. An Excel pivot table was used for the cross-streak test.

RESULTS AND DISCUSSION

Results

A total of 20 bacterial isolates from both soil and cast samples were tested for their antagonistic reactions through the agar diffusion test and cross-streak methods. There was a clear variation in the results from the zones of inhibition in diameters and on cross streak estimations across the isolates from the soil and earthworm cast samples.

Statistical analysis of the samples revealed that there was a significant difference in the rate of antimicrobial activity based on the isolation sources. Those isolates obtained from cast sources demonstrated a notably higher overall mean zone of inhibition (14.40mm) when they were compared to those derived from soil (2.33mm).

Table 1: Statistical Summary of Inhibition Zones (Mm) For Soil and Cast Isolates

Parameter	Soil Isolates	Cast Isolates
N	20	20
Mean (mm)	2.33	14.40
Variance	11.67	125.08
Std Dev	3.42	11.18

Note: The observed difference was statistically significant based on an independent samples t-test.

The result showed that the antimicrobial activity of the earthworm cast-derived bacterial isolates is significantly enhanced when compared to those isolates that were derived from soil sources. The distribution and inhibition zones, as shown in Figures 1 and 2, help to illustrate this by showing clear separations that exist between soil and cast isolate outcomes.

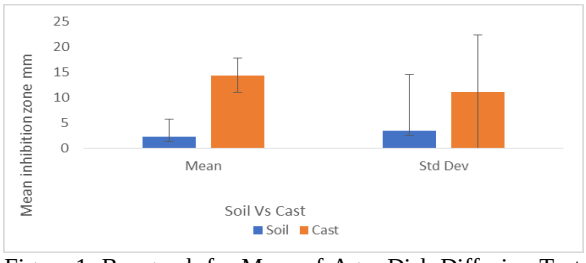

Figure 1: Bar graph for Mean of Agar Disk Diffusion Test Result for Earthworm Soil versus Cast Isolates

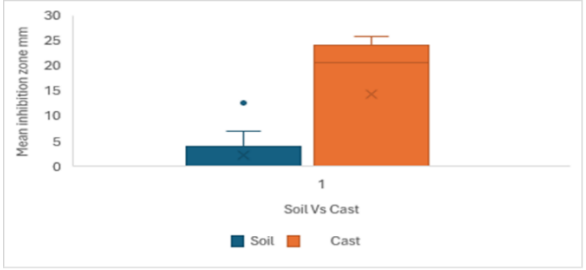

Figure 2: Box Whisker for the Mean of Agar Disk Diffusion Test Results for Earthworm Soil versus Cast Isolates

The genus with the most active antagonistic reaction, in the cast- derived sources were associated with the genera such as: *Bacillus* spp., *Streptomyces* spp., and *Pseudomonas* spp.

Table 2: Disk Diffusion Inhibition by Genus among Soil and Cast Isolates

Genus	Soil Activity	Cast Activity
Bacillus	Moderate	Very High
Streptomyces	Low–Moderate	High
Pseudomonas	Low–Moderate	High
Klebsiella	Low	Moderate
Enterobacter	Low	Moderate
Others	None	None

Although this group was also found in soil-derived sources, their antagonistic reactions showed moderate to low inhibitions against the tested pathogens. Isolates of *Bacillus* spp. and *Streptomyces* spp. from the cast samples mainly produced larger zones of inhibition. *Bacillus* spp. had the highest zone of inhibition across all isolates, with a mean inhibition zone, surpassing 23mm across all the pathogens being tested, such as *Escherichia coli*, *Salmonella* spp., and *Staphylococcus* spp., However, *Bacillus* spp., which were isolated from the soil, exhibited a moderate antagonistic activity, with inhibition zones below 15mm, occurring precisely within a range of 11–14 mm.

Moderate inhibition measurements were observed in some isolates, like *Klebsiella pneumoniae* and *Enterobacter aerogenes*, and their values also reflected less consistent and smaller zones of inhibition. In contrast to this observation, several isolates that included *Micrococcus* spp., *Corynebacterium* spp., *Neisseria sicca, and Morganella morganii* showed no detectable signs of inhibition reaction (0.0mm)

The outcome of the Agar well diffusion result, thus, indicates that antagonism is largely taxon-dependent and is significantly enhanced in the isolates that are obtained from the cast of earthworms when compared to those from soils.

Pathogen-Specific Response using both ANOVA tests within each group of soil or cast-derived isolates suggested that there was no significant difference when comparing how the isolates antagonised or inhibited the different test pathogens.

Table 3: **ANOVA for Inhibition by Soil Bacterial Isolates**

Source of variation	df	SS	MS	F	p-value
Between groups	2	0.281	0.141	0.012	0.988
Within groups	57	676.939	11.876		
Total	59	677.220			

Note: No significant difference ($p > 0.05$).

Table 4: ANOVA for Inhibition by Earthworm Cast Bacterial Isolates

Source of variation	df	SS	MS	F	p-value
Between groups	2	10.811	5.406	0.043	0.958
Within groups	57	7225.589	126.765		
Total	59	7236.400			

Note: No significant difference ($p > 0.05$).

A two-sample t-test assuming unequal variances was performed to compare the overall mean inhibition zones of the soil and cast- derived isolates. Notably, the mean inhibition zone for the cast isolates (14.40 ± 11.18 mm) was significantly higher than that of soil isolates (2.33 ± 3.42 mm). This difference was statistically significant ($t = -4.62$, $df = 23$, $p = 0.00012$). Such an outcome suggests that the antagonistic activity observed from both isolates is significantly enhanced in earthworm cast-derived bacterial isolates when compared to their soil-derived counterparts.

Table 5: **Independent Samples T-Test Comparing Inhibition Zones of Soil and Cast Isolates**

Variable	t-value	df	p-value	Remark
Mean scores	-4.62	23	<0.001	Significant

Note: Significant at $p < 0.05$.

Results from the cross-streak assay portrayed antagonistic activities, which were indicated by the presence of inhibition. This was quantified on a scale of 0 to 2. Results from the cross-streak test were consistent with those of the Agar well diffusion test, but displayed few differences in the interaction-based inhibition. The cross-streak assay supported the findings of the agar diffusion method by demonstrating a similar trend in antagonistic potentials across all the isolates.

Table 6: Cross-Streak Assay Inhibition Scores (Mean Values) Of Bacterial Isolates against Test Pathogens

Isolate	Source	*E. coli*	*Salmonella* spp.	*Staphylococcus* spp.	Mean Score
Acinetobacter baumannii	Cast	0.0	0.0	0.0	0.0
Bacillus spp.	Cast	2.0	2.0	2.0	2.0
Bacillus spp.	Soil	2.0	2.0	2.0	2.0
Corynebacterium spp.	Cast	0.0	0.0	0.0	0.0
Corynebacterium spp.	Soil	0.0	0.0	0.0	0.0

Enterobacter aerogenes	Cast	1.7	1.7	1.3	1.6
Enterobacter aerogenes	Soil	0.9	1.0	1.1	1.0
Klebsiella pneumoniae	Cast	2.0	2.0	2.0	2.0
Klebsiella pneumoniae	Soil	1.0	1.0	1.0	1.0
Micrococcus spp.	Cast	0.0	0.0	0.0	0.0
Micrococcus spp.	Soil	0.0	0.0	0.0	0.0
Morganella morganii	Cast	0.0	0.0	0.0	0.0
Morganella morganii	Soil	0.0	0.0	0.0	0.0
Neisseria sicca	Cast	0.0	0.0	0.0	0.0
Neisseria sicca	Soil	0.0	0.0	0.0	0.0
Proteus vulgaris	Cast	1.0	0.7	0.7	0.8
Proteus vulgaris	Soil	0.7	0.8	0.7	0.7
Pseudomonas spp.	Cast	2.0	2.0	2.0	2.0
Streptomyces spp.	Cast	2.0	2.0	2.0	2.0
Staphylococcus saprophyticus	Soil	0.0	0.0	0.0	0.0

Note: Cross-streak inhibition was scored on a scale from 0–2, where 0 = no inhibition, 1 = partial inhibition, and 2 = complete inhibition.

Cast-derived isolates, particularly *Bacillus* spp., *Streptomyces* spp., and *Pseudomonas* spp., consistently exhibited complete inhibition (score = 2.0) against the test pathogens. Soil-derived isolates showed weaker interactions, typically ranging from no inhibition (0.0) to moderate inhibition (~1.0). Observations from both tests showed that some isolates, such as *Micrococcus* spp., *Corynebacterium* spp., *Neisseria sicca*, and *Morganella morganii*, showed no inhibitory activity. While the agar diffusion test provided quantitative measurements of the zones of inhibition in diameter, the cross-streak method gave a qualitative indication of either the presence or the absence of the inhibitory interactions. There was no inhibition activity in the negative control in all the tests, signalling that the outcome observed antimicrobial inhibition was a result of the effect of the responsive bacterial isolates.

Table 7: Biochemical Test Results for Earthworm Cast and Soil Isolates

Isolate	Gram	Catalase	Oxidase	Indole	Citrate	Urease	Motility
Acinetobacter baumannii	-	+	-	-	+	-	-
Bacillus spp.	+	+	+	-	+	-	+
Corynebacterium spp.	+	+	-	-	-	-	-
Enterobacter aerogenes	-	+	-	-	+	-	+
Klebsiella pneumoniae	-	+	-	-	+	+	-
Micrococcus spp.	+	+	+	-	+	-	-
Morganella morganii	-	+	-	+	-	+	+
Neisseria sicca	-	+	+	-	-	-	-
Proteus vulgaris	-	+	-	+	+	+	+
Pseudomonas spp.	-	+	+	-	+	-	+
Staphylococcus saprophyticus	+	+	-	-	-	+	-
Streptomyces spp.	+	+	+	-	+	-	+

Biochemical test key
+ = Positive reaction
- = Negative reaction

Discussion

The results of the comparative antagonism in the sample sources indicate a significant enhancement of antagonistic potentials in the bacterial isolates from earthworm cast-derived samples compared to those derived from the surrounding soil. The notable difference in inhibition zones between soil and cast sources suggests that earthworm-mediated biological processes may be involved in enriching functionally active microbial populations. This emphasises a previous concept by Brown *et al.,* (2000) describing the roles of earthworms as ecosystem engineers, which modifies microbial structures and functions through their gut transit process, released as casts into soil (Sharma *et al.,* 2025; Gómez-Brandón *et al.,* 2011).

During the transit of organic matter, soil, and other contents in the guts of earthworms, the ingested contents may undergo some form of mechanical fragmentation, selective enrichments, and also enzymatic digestion. The unique physicochemical and biological conditions that exist in the guts of earthworms may often influence and favour microorganisms that are capable of surviving such competitive and nutrient-perturbed environments. This is mostly associated with the production of enhanced secondary metabolites and antimicrobial compounds by microorganisms (Khyade, 2018; Sharma *et al.,* 2025). The selectively enriched antimicrobial compounds produced by the microflora that is expelled out with the cast of earthworms may likely explain the significantly higher inhibition zones noticed in the cast isolates.

Comparison of both isolate sources indicates that bacterial species like *Bacillus* species exhibited the highest antagonistic activity as observed across both assay methods and sources. This consistency has been well documented in publications by Ongena and Jacques, (2008); Stein, (2005), suggesting their ability to produce antimicrobial compounds that may include lipopeptides, such as surfactin, fengycin, and iturin in large doses (Turin *et al.,* 2025). The existence of a dominant bacterium such as *Bacillus* spp. in the cast of

earthworm samples suggests that the gut of earthworms may internally provide a selectively spore-forming, antagonistic, and metabolically versatile bacterial species (Sharma *et al.,* 2025). These selectively enriched bacterial species are capable of adapting to the earthworm guts, cast, and soil sources, where they may be involved in the production of bioactive compounds.

Similar response was noted in *Streptomyces* spp., and *Pseudomonas* spp., regarding a potent and broad-spectrum inhibition from isolates derived from the cast samples. Bacterial species belonging to these genera have been widely recognized for their roles in both antibiotic production and for their ecological competitions in soil ecosystems (Kumar *et al.,* 2012; Devi & Serfoji, 2018; Tiwari *et al.,* 2015). Observations of such enhanced performance in the cast samples support the hypothesis that earthworm activity may contribute to the functional enrichment of microbial communities possessing high antagonistic potential (Yang *et al.,* 2024; Goswami, 2025).

Contrary to our observations, some isolates, including *Micrococcus*, *Corynebacterium*, *Neisseria sicca*, and *Morganella morganii*, did not show any form or detectable antagonism in both sample sources. This result suggests that antagonistic potential is highly taxon-dependent and may not uniformly be distributed across the microbial communities. Absence of zones of inhibition as found in these taxa may reflect some form of limited or insignificant biosynthetic capacity for the production of antimicrobial compounds or lower ecological competence when tested under certain conditions.

The reliability of the findings is supported by the concordance in the results observed between the cross-streak and the agar well diffusion assays. Agar well diffusion assays explicitly provide a quantitative measurement of the zones of inhibition in mm, while the cross-streak assay mainly confirms the presence or absence of inhibitory interactions between all the isolates and the test pathogens. Acclaimed differences between these two assays may be attributed to minor variations in their sensitivities based on the mechanisms being assessed. Others may be influenced by direct microbial confrontations and competition versus the rate of metabolite production.

A major finding from our results supports the concept that earthworm gut contents, which come out as casts, may function as potential hotspots for biologically active microbial species, which possess potential antagonistic capabilities (Sharma *et al.,* 2025; Khyade, 2018; Yang *et al.,* 2024). Implications from this finding benefit soil health systems with relevance to natural disease suppression and the development of microbial biocontrol systems for environmental sustainability. The significantly enhanced antagonistic activities of bacterial species that were observed in the cast-derived sourced isolates highlight the applied and ecological relevance of earthworm cast-associated microbiota.

Further research should be focused on using molecular techniques to characterize and identify, mostly, some of these specific groups of microorganisms with antimicrobial properties that are responsible for the noticeable inhibitory reactions. Identification of some potentially useful strains may be beneficial to soil management and environmental sustainability.

CONCLUSION

The significantly high rate of enzymatic activities and antagonism found in the earthworm casts suggests that the gut of earthworms may have selectively transited enriched antagonistic bacterial species that are inhibitory against pathogens. This significant increase in their inhibitory activities provides technical validity, justifying the notion that vermicasts can be used as a tool for bioprospecting of some novel antimicrobial compounds. Earthworm microbiomes from both guts and cast sources can be exploited as natural solutions for environmental biosecurity.

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