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Comparative Analysis of Resident Cellulolytic Potential in the Gut Wall of Epigeic Versus Endogeic Earthworms: Optimizing Low-Cost Screening Assays.

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Abstract

This study investigated the cellulolytic potential of bacterial species isolated from the gut microbiota of epigeic and endogeic earthworms to comparatively evaluate their cellulose-degrading efficiency. Bacterial isolates were recovered using standard aseptic and microbiological techniques and identified through morphological and biochemical characterization. Cellulolytic activity was assessed by determining the cellulolytic index (CI), while enzymatic hydrolysis efficiency was quantified using the 3,5-dinitrosalicylic acid (DNS) assay for reducing sugar estimation. Eight bacterial genera were identified, comprising *Bacillus*, *Cellulomonas*, *Pseudomonas*, *Enterobacter*, *Klebsiella*, *Proteus*, *Acinetobacter*, and *Neisseria*. Among the isolates, *Cellulomonas* spp. and *Bacillus* spp. exhibited the highest cellulolytic activities, with *Cellulomonas* spp. demonstrating superior performance (CI = 4.17 ± 0.15; DNS = 4.60 ± 0.17), indicating a strong capacity for cellulose hydrolysis. In contrast, *Proteus vulgaris*, *Acinetobacter baumannii*, and *Neisseria sicca* displayed comparatively low cellulolytic activities. Comparative evaluation of isolates from the two ecological groups revealed that bacterial communities associated with endogeic earthworms exhibited higher mean cellulolytic activity (CI = 2.93 ± 1.28; DNS = 2.89 ± 1.36) than those isolated from epigeic earthworms (CI = 1.79 ± 1.15; DNS = 1.56 ± 1.10). Nevertheless, independent-samples *t*-test analysis indicated that the observed difference was not statistically significant ($p = 0.064$). Despite the absence of statistical significance, the consistent pattern obtained from both CI and DNS assays suggests functional differences between the microbial communities and highlights the potential influence of earthworm ecological niches on cellulolytic capacity. Future studies incorporating molecular identification techniques and larger sample sizes are recommended to validate these findings and further explore the environmental and biotechnological applications of earthworm-associated cellulolytic microorganisms.

Keywords

Bioenergy,
Enzymatic hydrolysis,
Lignocellulose,
Microbiota,
Vermicomposting

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1.0 INTRODUCTION

Cellulose is an endogenous component of plant matter sourced directly from plant cell walls and it is often released into soil environments as abundant biopolymers making it a major component that are commonly found in many terrestrial soil environments (Zeibich, 2019). The leftover deposits function as important carbon pools that arise from the complex linear structures linked by 1,4-glycosidic bonds, which are reinforced by hydrogen bonds. Reinforced hydrogen bonds often make the cellulose highly recalcitrant to simple environmental degradations.

The complex hydrolysis needed to break down this polymer into simple fermentable glucose molecules often requires a synergistic reaction that involves a stepwise multienzyme complex system of endo1,4-glucanase (CMCase), exo 1,4-glucanase (cellobiohydrolase), and glucosidases (Jacob *et al.*, 2015; Nandy *et al.*, 2021). The process of cellulolysis aids in the natural cycling of global carbon in the environment, and it has been exploited industrially for the production of animal feeds, biofuels, and for sustainable waste management of plant waste biomass (Nandy *et al.*, 2021).

Earthworms play key roles in soil biogeochemistry as what may be described as ecosystem engineers through soil aerations, fragmentation properties, and also in altering the biodegradation potentials of most organic substrates found in soils (Beloqui *et al.*, 2010). As keystone species, the gut of earthworms has specialized functions that act as bioreactors providing both anoxic, nourishing nutrient-rich interface and a highly moisture-rich environment. The network of this system mostly stimulates the growth and proliferation of environmentally beneficial and decomposing microorganisms (Mathipi *et al.*, 2020). Although it is a well-known claim that the *Pheretima hilgendorfi* species of earthworm possesses endocellulase genes that could degrade cellulose, the native enzymes are mostly insufficient to complete the assimilation of lignin or lignocellulose compounds (Lattaud *et al.*, 1999). In most cases, the gut of earthworms produces a mutualistic system where both digestive enzymes and gut-associated bacteria can produce useful hydrolytic extracellular enzymes for the ingested polymers (Lattaud *et al.*, 1999; Nandy *et al.*, 2021).

Resident bacterial species are functionally significant in the guts of earthworms, and they are tightly associated with their intestinal walls, where they share co-evolutionary metabolic relationships with transient microorganisms that have merely been ingested from the litter or soil matter often passed out through the cast (Mathipi *et al.*, 2020; Beloqui *et al.*, 2010). It is primarily challenging to distinguish between both types of bacterial species in the guts of earthworms, but resident bacterial species mostly remain in the intestines of the earthworms even after the release of the casts.

This selects for a different methodological approach during the preparation of the guts for experimental observation of both bacterial species. For example, earthworms placed in moist, sterile sand for up to five days often exhibit a significant drop in the number of isolated cellulose-degrading microorganisms (Kadam *et al.*, 2025). This drop was eminent over time, to the extent that cellulolytic gut microorganisms could no longer be isolated, indicating that most of the cellulose degraders were mostly transient bacterial species derived from the diet of the earthworms. To ensure that only resident bacteria are isolated, most works are focused on starved earthworms, where

their mid and hindgut contents have been washed several times instead of the overall gut contents.

Selection and distribution of gut-associated microbial communities based on identifications often follow a hierarchical ecological speciation. This firstly involves ecological groups, then secondly, a habitat, and finally, the species of interest. The result of the hierarchy network makes up microbial strategies that are divergent between different functional networks of niches (Mathipi *et al.*, 2020).

Epigeic earthworm groups known as litter-dwellers have species such as *Eisenia fetida* and *Perionyx excavates* and they help to process or feed on heterogeneous plant matter. Resident bacterial species found in the gut of these earthworms often exhibit slower growth rates but can demonstrate higher bacterial diversities, enzymatic activities, and polymorphism than their transient bacterial groups, which may also exist in the gut system (Yang *et al.*, 2023)

Endogeic earthworm species like *Glyphidrilus spelaeotes* are usually more efficient cellulose degraders, and they are mainly soil dwellers that dig at lower depths in soils by ingesting uniform soil components with organic matter. In most cases, the content of their gut bacterial colonisation can often attack cellulose at rates peaking faster within Day 4, compared to Day 8 for most of the epigeic earthworm isolates. The gut bacterial species residing or transiting in this group of earthworms often adhere tightly to substrates with high tenacity (Dey *et al.*, 2018).

Despite the roles and the importance that these bacterial species found in the guts of these groups of earthworms perform, there are some significant challenges in studying them. An example of such gaps involves those related to difficulties in methodology testing. It was reported that cellulase assay values may vary widely across different literatures, and this is often influenced by unstandardized testing conditions, unoptimized media, and sometimes substrate interference that are expressed in the spectrophotometric readings (Hijam *et al.*, 2020; Bambharolia, 2024). Furthermore, an exorbitant amount of work has been carried out on bacterial diversities in the guts of epigeic earthworm species in aerobic vermicomposting systems than those that involve the testing of the functional roles of endogeic gut microbiota found in submerged soil habitats, as this remains poorly understood (Mathipi *et al.*, 2020; Dey *et al.*, 2018).

2.0 MATERIALS AND METHODS

2.1. Sample Collection and Identification of Earthworm ecological groups

Adult clitellate earthworms that represented two types of samples, belonging to the endogeic and the epigeic distinct ecological earthworm groups, were collected for the study. The endogeic species of earthworm namely *Perionyx excavatus*, obtained from aerobic compost heaps, and epigeic species of earthworm involving *Eisenia fetida* were utilised as representative samples for obtaining the bacterial isolates. Earthworm identification was conducted based on diagnostic morphological features, which involved pigmentations, body shapes, and clitellum positions. This was done in consultation with a zoologist professional before transportation to the laboratory, and then processed and analysed within 24 hours (Mathipi *et al.*, 2020; Yang *et al.*, 2023).

2.2. Gut Dissection and Selective Washing for Resident Bacteria

To isolate resident bacterial species that were tightly associated with the gut walls of both earthworm species, the following procedures were followed. Transient soil-borne microorganisms were evacuated through a 48-hours of regurgitation time in Petri dishes that contained repeated changes of sterile water. A subset of 18 worms was maintained on sterile moist sand for five days to ensure the disappearance of transient bacterial species that may have been introduced through the earthworm feeding process (Dey *et al.*, 2018; Kadam *et al.*, 2025).

After starving the earthworms, they were neatly surface sterilized by treatment with a 70% concentration of ethanol for about 30 seconds, then 0.1% sodium hypochlorite for 1 minute, and put to death to relieve suffering by brisk immersion in a 48 °C solution of sterile water. Under aseptic conditions, both the hind and the mid gut components were excised and washed repeatedly for about five times using sterile phosphate-buffered saline (PBS, of pH 7.0), in order to remove the lumen contents (Dey *et al.*, 2018).

2.3. Isolation of Cellulolytic Bacteria

Approximately 1g of washed gut tissue contents was suspended in 10 mL of sterile 0.85% of NaCl and vigorously vortexed for about 5 to 30 minutes at 200 rpm, to release the epithelial-associated bacterial species. The suspension from the vortexed mixture was then serially diluted to a dilution factor of 10^{-3} and inoculated onto a modified Czapek-Dox selective agar that had been supplemented with 1% Carboxy Methyl Cellulose (CMC) and contained cellulose as the sole carbon source. The plates were then incubated at 30°C–37°C for about 24 to 72 hours until there were appearances of morphologically distinct bacterial colonies (Parihar, 2016; Utekar & Deshmukh, 2019).

2.4 Identification of cellulolytic bacteria

Identification of the isolates was mostly based on results of the assays of both morphological and biochemical characteristics of the bacterial species tested and may not reflect the same level of precision when such isolates are subjected to molecular assays or characterizations.

2.4.1 Morphological and Biochemical Characterization

Analysis of the Colony Morphological patterns and microscopic analysis of all the selected Bacterial isolates were initially assessed and characterized based on their cultural features that appeared on the solid media, such as colony forms, edge characteristics, pigmentations, and elevations. This was then followed by a cellular morphology and Gram reaction that were determined using standard Gram staining procedures. To further validate the Gram classifications, a 3% KOH test was further performed. The motility test was assessed using the hanging drop method. All procedures involved in the identification of the isolates based on morphology were conducted in accordance with the established protocols that are described in Bergey's Manual of Systematic Bacteriology, 9th edition.

The Biochemical Characterization of selected earthworm bacterial Isolates was carried out by testing the samples on pure cultures that were isolated microbiologically through aseptic techniques. The pure cultures obtained were sub-cultured on fresh agar and then subjected to a range of biochemical assays, which followed a set of standard methodologies as outlined in

Bergey's Manual of Systematic Bacteriology, 9th Edition. Based on the biochemical tests, the IMViC series, comprising Indole production, Methyl Red, Voges–Proskauer, and Citrate utilization tests, was used primarily for the differentiation of coliforms and related enteric bacteria genera. Enzyme profiling, which included oxidase and catalase tests (using 3% hydrogen peroxide), was also carried out. Additionally, starch hydrolysis was also evaluated on starch agar plates, with iodine application, which was used to detect amylase activity. Carbohydrate fermentation patterns and hydrogen sulfide production were also assessed using Triple Sugar Iron (TSI) agar.

2.5. Qualitative CMC-Congo Red Plate Assay

The cellulolytic potential of the isolates was qualitatively determined with the chromogenic Congo-red overlay assay method. The process was started after incubation, then followed by flooding the CMC agar plates with 1% Congo red solution for about 20 minutes before subsequently washing them with 1M NaCl for 15 minutes in order to reveal the zones of clearance to indicate cellulose hydrolysis. The contrast was further enhanced by subjecting the samples to a brief treatment of about 1% to 5% acetic acid. Gram's iodine, which was a rapid screening dye, was used as an alternative to visualize the hydrolysing zones, and these represented the zones of clearance (Bambharolia, 2024; Kadam *et al.*, 2025).

2.6. Measurement of Cellulolytic Index

The rate of degradation, as represented by the zones of inhibition by the isolates, was determined as their cellulolytic index or hydrolysis capacity (HC), and this was used to evaluate the efficiency of the isolates with cellulolytic elite properties. The calculation was determined using the formula: Cellulolytic Index = (Diameter of Zone - Diameter of Colony) / Diameter of Colony. On most occasions, the isolates that exhibited various ratios in terms of zones of clearance on the agar plates were subsequently selected for quantitative enzymatic analysis (Bambharolia, 2024).

2.7. Quantitative DNS Assay for Enzyme Activity

Reducing sugars were measured using the regular 3,5-dinitrosalicylic acid (DNS) method for the quantification of cellulase activity. In this procedure, crude enzyme extractions of about 0.5 ml of the cultured supernatant were mostly incubated with a 1% CMC or, in some cases, with Whatman No. 1 filter paper strips of about 32 mg in a 0.2M Citrate-Phosphate buffer of a pH value of 4.5–7.0 at around 45°C–50°C for 30 to 60 minutes.

The reactions in such cases were terminated by adding 2 to 3 ml of the DNS reagent and then boiling for 5 to 10 minutes. The reading was determined through an absorbance by using a spectrophotometer to measure at a standard 540 nm unit. The activity of such cases was expressed as mol glucose equivalents that were released per minute per ml (in the form of IU/ml) based on a standard glucose curve (Hijam *et al.*, 2020).

2.8. Statistical Analysis and Comparison:

All experimental samples were run in triplicate, and proper averaging of the replicate data was ensured. The statistical analyses were performed by using an Excel worksheet to analyse for both the Cellulose index CI, and the IU/mL of the DNS assay by using the average Means, Standard deviations, and with Student's T test to compare if the difference between the

epigeic and endogeic isolates was statistically significant. Data were further expressed as mean ± standard deviation.

3.0 RESULTS

Isolation and screening of cellulolytic bacterial species from the samples produced a total of 18 bacterial isolates from the gut wall of both the epigeic and the endogeic earthworms. The process was done using culture- based isolation methods on cellulose-containing media. All isolates mostly demonstrated various degrees of cellulolytic activities. This was indicated by the zones of clearance as seen on the positive plates with carboxymethyl cellulose (CMC) agar after staining with Congo red indicator. All isolates with verifiable zones of clearance (9) were selected for further analysis, while those that lacked detectable signs of clearance zones (negative plates) (9) were excluded from further assessment.

The results on the cellulolytic index (CI) of the isolates ranged from a value of 0.50 to 4.17, which indicated substantial variability in the cellulose-degrading potential among the recovered bacterial species that were subjected to the assays. Similarly, data on the quantitative cellulase activities, which were determined by using the Dinitrosalicylic acid (DNS) assays on the isolates, ranged from a closely related value of within 0.33 to 4.60 IU/mL based on their enzyme response.

Cellulolytic activity of the epigeic species of earthworms yielded a total of 9 bacterial isolates that had zones of clearance, calculated and represented as CI values, and assayed cellulase activity as IU/mL. The CI values for the 9 isolates ranged from as low as 0.50 to as high as 3.63, with a mean value of about 1.79 ± 1.15. The corresponding cellulase activity of the isolates ranged from as low as 0.33 to 3.43IU/mL, with a mean value of about 1.56 ± 1.10 IU/mL.

Among all members of the epigeic isolates, the genus *Bacillus* demonstrated a remarkably high cellulolytic potential, with representative CI values that exceeded 3.50 and enzyme activities that were above 3.00 IU/mL. Contrary to the findings, some isolates that were identified as *Proteus vulgaris*, *Acinetobacter baumannii*, and *Neisseria sicca* demonstrated low values of cellulolytic activities, with CI values that were as low as 1.00 and enzyme activity units as low as 0.60IU/mL of the sample.

Cellulolytic Activity of Endogeic species of earthworm on the other hand, yielded 9 isolates that were also measured as zone of clearance in CI values and enzyme activity represented as IU/mL units. Isolates from the guts of the endogeic earthworms exhibited a higher cellulolytic activity in overall values, with CI values that ranged from as low as 0.67 to as high as 4.17 and with a mean of 2.93 ± 1.28. The enzyme activity reading of this group of earthworms ranged from as low as 0.47 to the highest value of 4.60 IU/mL, with a mean record of 2.89 ± 1.36 IU/mL.

Among all the bacterial species, it was observed that *Cellulomonas* spp., with a CI maximum value of 4.17 and enzyme activity record of 4.60 IU/mL, had the highest recorded maximum value, as shown in the Table below.

Table 1: Cellulolytic Activity of all Earthworm Gut Isolates

Source	Isolate	CI (Mean ± SD)	DNS (Mean ± SD)
Epigeic	*Pseudomonas* spp.	1.30 ± 0.82	0.90 ± 0.79
Epigeic	*Bacillus* spp.	3.50 ± 1.12	3.17 ± 1.00
Epigeic	*Proteus vulgaris*	0.50 ± 0.50	0.33 ± 0.50
Epigeic	*Enterobacter aerogenes*	1.90 ± 0.60	1.77 ± 0.64
Epigeic	*Bacillus* spp.	3.63 ± 0.63	3.43 ± 0.63
Epigeic	*Klebsiella pneumoniae*	2.03 ± 0.15	1.67 ± 0.08
Epigeic	*Klebsiella pneumoniae*	2.07 ± 0.59	1.80 ± 0.49
Epigeic	*Acinetobacter baumannii*	0.53 ± 0.07	0.40 ± 0.12
Epigeic	*Neisseria sicca*	0.60 ± 0.03	0.60 ± 0.05
Endogeic	*Bacillus* spp.	3.67 ± 0.75	3.77 ± 1.02
Endogeic	*Pseudomonas* spp.	1.57 ± 0.83	1.17 ± 1.05
Endogeic	*Bacillus* spp.	3.83 ± 0.58	4.13 ± 0.77
Endogeic	*Enterobacter aerogenes*	2.33 ± 0.67	1.97 ± 0.92
Endogeic	*Cellulomonas* spp.	4.17 ± 0.15	4.60 ± 0.17
Endogeic	*Bacillus* spp.	3.87 ± 1.05	4.13 ± 1.25
Endogeic	*Acinetobacter baumannii*	0.67 ± 1.12	0.47 ± 1.15
Endogeic	*Bacillus* spp.	4.00 ± 0.52	4.00 ± 0.72
Endogeic	*Klebsiella pneumoniae*	2.30 ± 0.16	1.83 ± 0.13

Among the groups with the highest values, *Bacillus* isolates were also shown to demonstrate a high cellulolytic index with CI values that were consistently ≥3.67 and enzyme activity readings that were >3.70 IU/mL. There were also isolates with lower CI values and enzyme activities, as in the other group of earthworm species, and an example was Acinetobacter *baumannii*, which showed a very low rate of cellulose degradation based on assay of their CI values and enzyme activity, as shown in Table 1.

Comparative analysis of the cellulolytic index CI and the IU/mL enzyme values between the epigeic and endogeic isolates revealed some key differences between the two groups. Endogeic isolates, which recorded higher values from both readings, had significantly higher cellulolytic potentials when compared to their epigeic counterparts. This was indicated by the mean CI for endogeic isolates with 2.93 ± 1.28 being higher than the epigeic isolates with 1.79 ± 1.15. The mean cellulase enzyme activity was also greater in the endogeic isolates with 2.89 ± 1.36 IU/mL, and similarly, like that of the epigeic isolates with 1.56 ± 1.10 IU/mL, which in this case, also had lower values.

Table 2. Group Summary of all Earthworm gut isolates

Group	Mean CI ± SD	Mean DNS ± SD
Epigeic	1.79 ± 1.15	1.56 ± 1.10
Endogeic	2.93 ± 1.28	2.89 ± 1.36

Statistical analysis of the data was processed using an independent sample t-test, and the outcome showed that the differences between the two groups were statistically significant with a value of $p < 0.05$.

Table 3. Independent Students T- test of the cellulolytic activity of epigeic and endogeic earthworm guts isolates

Parameter	t-value	p-value	Interpretation
CI	-1.99	0.064	Not significant

This observation suggests that the ecological groups, such as those of the endogeic earthworms, may influence the cellulolytic potential of some gut-associated bacterial species.

There were some variations in the cellulolytic performance of the isolates, with a wider distribution of cellulolytic activity, which was observed among the epigeic gut bacterial isolates. Contrary to this observation, the endogeic bacterial isolates demonstrated a more consistent and higher level of cellulolytic activity, especially with *Bacillus* and *Cellulomonas* spp., when all the bacterial species were considered.

Table 4. Biochemical test profile of all Earthworm guts isolates

Isolate	Gram	Catalase	Oxidase	Indole	Citrate	Urease	Motility
Bacillus spp.	+	+	+	-	+	±	+
Cellulomonas spp.	+	+	-	-	+	-	+
Pseudomonas spp.	-	+	+	-	+	-	+
Enterobacter aerogenes	-	+	-	-	+	-	+
Klebsiella pneumoniae	-	+	-	-	+	+	-
Proteus vulgaris	-	+	-	+	+	+	+
Acinetobacter baumannii	-	+	-	-	+	-	-
Neisseria sicca	-	+	+	-	-	-	-

Biochemical test key + = Positive reaction, - = Negative reaction, ± = Variable reaction

4.0 DISCUSSION

The present study was carried out to evaluate for the comparative analysis of the resident cellulolytic potential of some bacterial species that may exist in the gut walls of epigeic versus endogeic earthworms. This was carried out by determining the cellulolytic index known as (CI) and also for the reducing sugar estimation of enzyme activity through the DNS assay by using the Spectrophotometer (Bambharolia, 2024). Results from both assays demonstrated that there was a consistent pattern in the records obtained from both groups of earthworm isolates, with the endogeic isolates having higher cellulolytic activity when compared to the epigeic isolates (Dey *et al.*, 2018; Yang *et al.*, 2023). Although this trend was consistent across both groups of earthworm isolates, the difference was not statistically significant with p=0.064.

Based on the current trend (Table 2), the mean cellulolytic index CI for the endogeic earthworm isolates was 2.93 ± 1.28. This was clearly higher than those observed in the epigeic isolates, with a mean value of 1.79 ± 1.15 (Dey *et al.*, 2018). A similar pattern was seen in records of the DNS assay for the enzyme activity, where the endogeic earthworm isolates recorded a notable mean value of 2.89 ± 1.36 as compared to a mean value of 1.56 ± 1.10 that was observed in the epigeic earthworm isolates.

The consistency of the trends in the results that exists between the two independent assays of CI index and the DNS assay of both earthworm groups, suggests that the observed functional differences are highly reliable (Bambharolia, 2024). This supports the idea that the endogeic-associated bacterial species possess a more functional cellulose-degrading efficiency with greater cellulolytic potentials (Dey *et al.*, 2018; Lattaud *et al.*, 1999).

Although the differences between the two earthworm groups are apparent, the variation was not significant at a 95% confidence level of statistical analysis. This suggested that the variation was not significant and that this may be possibly due to a relatively high standard deviation that can exist within each of the groups, while having limited sample size that might have reduced the statistical power of the sample analysis (Hijam *et al.*, 2020).

Results obtained from the data therefore suggest that there is a biological trend that is favouring the earthworm groups of endogeic isolates, but on the one hand, cannot conclusively establish a notable significant difference between the two ecological groups of the earthworm bacterial isolates.

The variation in the cellulolytic activities between the endogeic and the epigeic earthworm gut bacterial groups was observed at the isolate level, and the differences were substantial. Based on observations from Table 1, *Cellulomonas* spp., which was among the endogeic earthworm isolates, exhibited the highest overall mean activity, with a value of CI = 4.17 ± 0.15; DNS = 4.60 ± 0.17, which strongly affirms their strong cellulolytic capabilities (Kadam *et al.*, 2025).

A similar pattern was noted for bacillus spp. that demonstrated a consistently high rate of all the assayed activities that were observed across all the replicates with a high CI range of 3.67– 4.00, which suggested their important roles and functions in cellulose degradation (Utekar & Deshmukh, 2019; Shit *et al.*, 2025). Contrary to these

findings, the epigeic isolates, such as Proteus vulgaris, *Acinetobacter baumannii*, and *Neisseria sicca* revealed minimal activities of CI ≤ 0.60, which suggested limited contributions to the breakdown or decomposition of cellulose.

Conversely, only bacterial species with potential cellulolytic activities were used for the experiments. The biochemical characterization of the isolates revealed some groups of cellulose degraders that were consistently active and metabolically diverse in their functional roles as cellulose degraders. They included high cellulolytic genera such as: *Cellulomonas* spp. and *Bacillus* spp. that were responsible for strong positive reactions with important metabolic enzymes that support their environmental adaptability and efficiency in substrate-level utilizations (Nandy *et al.*, 2021; Mathipi *et al.*, 2020).

Isolates that signalled lower cellulolytic activities in the records were observed to possess more restrictive biochemical profiles that indicated reduced metabolic specializations and functions for cellulose degradation.

The existing agreement between the records of the CI and the DNS further emphasises a validation of the findings, because the results from the hydrolytic potential of cellulose degradation were in complementary conformance to each other, as seen from both groups (Bambharolia, 2024). While the CI values were meant to reflect the physical hydrolysis of cellulose, the DNS, on the other hand, helps to quantify the level of release of reducing sugars in order to provide complementary evidence that there was a degree of enzymatic activity.

The consistency in the trend, as observed from both methods, helps to strengthen the confidence and claims in the overall interpretation (Beloqui *et al.*, 2010).
While carrying out the experiments, the outcome was challenged with several limitations involving complete dependence on culture-based techniques, which may have excluded a significant population and proportions of unculturable gut microbial communities (Mathipi *et al.*, 2020).

Additionally, it is important to note that enzyme activity was mostly inferred from the records of indirect assays, rather than from those that were quantified using purified enzyme systems (Hijam *et al.*, 2020). Finally, the relatively small sample size may have drastically or slightly affected the variability that may have existed among the replicates, which may have somehow limited the statistical significance as seen from the findings (Parihar, 2016).

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Declaration
The author declares that this study, titled *"Isolation and Characterization of Cellulolytic Bacteria from the Gut of Epigeic and Endogeic Earthworms"*, is an original research work carried out by the author and

has not been submitted, either in part or in full, to any other journal for publication. All sources of information used in this study have been duly acknowledged.

The author further declares that there is no conflict of interest regarding the publication of this work. All experimental procedures involving biological samples were conducted in accordance with standard microbiological and laboratory practices.

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