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Culturable Soil Microbial Populations and their Potential Relevance to Xenobiotic Bioremediation in and around Don Bosco College of Agriculture, Sagayathottam, Ranipet District, Tamil Nadu, India

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A B S T R A C T

Soil microorganisms play a role in decomposition, nutrient cycling, and the transformation of organic chemical residues. The present investigation characterized culturable bacterial, fungal and actinobacterial populations in soils representing ten land-use sites in and around Don Bosco College of Agriculture (DBCA), Sagayathottam, Ranipet District, Tamil Nadu, and assessed their potential relevance as indigenous microbial resources for future xenobiotic-biodegradation studies. Methods: Composite soil samples were collected from 0–15 cm depth and serially diluted. Bacteria, fungi and actinobacteria were enumerated by spread plate technique on Nutrient Agar, Rose Bengal Agar and Ken Knight's Agar, respectively. Three replications were evaluated for each site, and site effects were assessed by analysis of variance. Results revealed that Bacterial populations highest value 14.98 × 10^{-5} CFU g^{-1} soil, recorded in the sapota field. Fungal populations recorded 24.55 × 10^{-2} CFU g^{-1} soil, which is highest value. Actinobacterial populations ranged from 6.46 to 15.92 × 10^{-3} CFU g^{-1} soil, with the highest value in the crop cafeteria and the lowest in the sapota field. A predominant fungal isolate was morphologically identified as *Aspergillus niger*. Culturable microbial populations differed substantially among land-use sites, indicating site-specific microbial profiles. The findings provide a preliminary basis for selecting indigenous microorganisms for future xenobiotic-degradation screening; however, direct degradation of specific xenobiotic compounds was not demonstrated in this study.

Introduction

Xenobiotics are chemical substances that are foreign to a biological system and are not normally produced or expected to occur in that system. They are introduced into the environment through agricultural, industrial, pharmaceutical, domestic and urban activities. Agricultural soils may receive synthetic chemicals through repeated use of pesticides, herbicides, fungicides and other crop-protection products, while industrial and urban activities contribute petroleum hydrocarbons, synthetic dyes, solvents, plastics, pharmaceuticals and other chemical contaminants. Depending on their chemical structure and environmental conditions, some of these compounds can persist in soil and water and affect biological processes (Miglani *et al.,* 2022; Štefanac *et al.,* 2021).

Persistent chemical contaminants can influence soil biological properties, microbial community structure, nutrient cycling and ecosystem functioning. Their persistence is influenced by characteristics such as aromaticity, hydrophobicity, molecular structure and interactions with soil organic matter, which can reduce bioavailability and slow microbial transformation (Li *et al.,* 2024). Agricultural soils are particularly relevant because repeated chemical inputs can create chronic exposure of soil microbial communities to synthetic compounds (Wang *et al.,* 2022).

Microorganisms are central to the transformation of many organic compounds in soil. Bacteria, fungi and actinobacteria possess diverse metabolic capabilities and enzymes that can participate in oxidation, reduction, hydrolysis, dehalogenation, biotransformation, co-metabolism and mineralization. Bacterial genera such as *Pseudomonas, Bacillus, Rhodococcus, Arthrobacter* and *Acinetobacter* have been associated with transformation of various organic pollutants, while fungi can produce extracellular oxidative enzymes such as laccases and peroxidases that act on structurally complex compounds (Arora, 2020; Magan *et al.,* 2010). Actinobacteria are also important soil decomposers and can produce hydrolytic and oxidative enzymes involved in the transformation of recalcitrant organic substrates.

The occurrence and abundance of culturable microbial groups vary with land use and soil environmental conditions. Moisture, organic inputs, plant-root activity, aeration, cultivation practices and chemical inputs can influence microbial abundance and community composition. Consequently, characterization of indigenous microbial populations is an important preliminary step in identifying microbial resources for subsequent bioremediation investigations. Culture-dependent enumeration, however, represents only the culturable fraction of the soil microbiome and should not be interpreted as a measure of total microbial abundance.

Materials and Methods

Experimental Location

The investigation was conducted in the Department of Agricultural Microbiology, Don Bosco College of Agriculture (DBCA), Sagayathottam, Ranipet District, Tamil Nadu, India. The experimental location was reported as approximately 13°02′ N latitude and 79°77′ E longitude, at an altitude of approximately 91 m above mean sea level.

Site Selection and Soil Sampling

Ten sampling sites representing different land-use conditions were selected in and around the DBCA campus and nearby agricultural areas. Sampling locations were selected to represent differences in cropping systems, land use, moisture conditions and management history. Composite soil samples were collected from a depth of 0–15 cm. Surface litter, stones, roots and plant debris were removed before sampling. Soil was collected from approximately 5 points at each location using a sterile soil auger or trowel. Equal quantities from individual sampling points were mixed thoroughly in a sterile tray to obtain a representative composite sample. Approximately 500 g to 1 kg of homogenized soil was transferred into sterile, labelled polyethylene bags and transported for microbiological analysis.

Sterilization and Culture Media

Glassware was sterilized in a hot-air oven at 160–170°C for 2 h. Culture media and other autoclavable materials were sterilized at 121°C and 15 psi for 15–20 min. Nutrient Agar was prepared at 28 g L^{-1} and adjusted to approximately pH 7.0 ± 0.2 for bacterial enumeration. Rose Bengal Agar was prepared at 31.5 g L^{-1} and adjusted to approximately pH 5.6 ± 0.2 for fungal enumeration. Ken Knight's Agar was prepared at 39 g L^{-1} and adjusted to approximately pH 7.2 ± 0.2 for actinobacterial enumeration. Prepared media were checked for sterility before inoculation.

Serial Dilution and Microbial Enumeration

Ten grams of soil were suspended in 90 mL of sterile double distilled water and shaken at approximately 150 rpm for 20–30 min. Serial ten-fold dilutions were subsequently prepared. Aliquots of 0.1 mL from appropriate dilutions were plated in triplicate using the spread-plate technique. Bacteria were enumerated on Nutrient Agar at 37 ± 2°C for 24–48 h, fungi on Rose Bengal Agar at 28 ± 2°C for 3–5 days, and actinobacteria on Ken Knight's Agar at 28 ± 2°C for 5–7 days.

Discrete colonies were counted after incubation and expressed as colony-forming units (CFU) using the appropriate dilution factor and plated volume. Pure cultures were obtained by repeated streaking and maintained on agar slants at 4°C.

Fungal confirmation

Fungal isolates were examined using colony morphology and available microscopic characteristics. The predominant isolate was identified morphologically as *Aspergillus niger*. The identification is considered morphological rather than definitive molecular species confirmation.

Statistical Analysis

The ten-site comparison was analysed as a Completely Randomized Design with three replications per site. The supplied results indicate that microbial population data were transformed using logarithmic transformation before analysis of variance. Analysis of variance was used to test the effect of sampling site. Where significant differences were detected, Duncan's Multiple Range Test (DMRT) was used for mean separation at P = 0.05.

Means followed by different letter groups differ according to Duncan's Multiple Range Test (DMRT) at P = 0.05. Population values are expressed as CFU × 10^{-2} g^{-1} soil.

Results and Discussion

Bacterial Population

Bacterial populations differed significantly among the ten sampling locations and ranged from 6.54 to 14.98 × 10^{-5} CFU g^{-1} soil. The highest population was recorded in the sapota field (S6; 14.98 × 10^{-5} CFU g^{-1} soil), followed by the DBCA wetland (S7; 13.89 × 10^{-5} CFU g^{-1} soil). The lowest population occurred in the mulberry field (S4; 6.54 × 10^{-5} CFU g^{-1} soil). The site effect was highly significant (F = 17.8473; P < 0.01), with a coefficient of variation of 4.52%.

Fungal population

Fungal populations also varied among sampling sites, ranging from 9.25 to 24.55 × 10^{-2} CFU g^{-1} soil. The DBCA wetland (S7) recorded the highest fungal population (24.55 × 10^{-2} CFU g^{-1} soil), followed by the tomato field (S1; 21.65 × 10^{-2} CFU g^{-1} soil). The lowest fungal population was observed in the sapota field (S6; 9.25 × 10^{-2} CFU g^{-1} soil). The site effect was significant at the 1% level (F = 4.7576; P < 0.01).

Actinobacterial Population

Actinobacterial populations ranged from 6.46 to 15.92 ×10^{-3} CFU g^{-1} soil. The highest population occurred in the crop cafeteria (S3; 15.92 × 10^{-3} CFU g^{-1} soil), followed by Takkolam (S9; 15.32 × 10^{-3} CFU g^{-1} soil) and the orchard (S5; 15.09 × 10^{-3} CFU g^{-1} soil). The lowest population was recorded in the sapota field (S6; 6.46 × 10^{-3} CFU g^{-1} soil). The site effect was highly significant (F = 51.0550; P < 0.01), representing the strongest site effect among the three microbial groups.

Comparative Microbial Profile

The three microbial groups displayed contrasting spatial patterns. The sapota field had the highest bacterial population but the lowest fungal and actinobacterial populations. The DBCA wetland had the highest fungal population and a high bacterial population but a comparatively low actinobacterial population. The crop cafeteria recorded the highest actinobacterial population and a comparatively high fungal population.

The present investigation demonstrated substantial site-associated variation in culturable bacterial, fungal and actinobacterial populations. Such variation is consistent with the heterogeneous nature of soil environments, where microbial abundance can be influenced by nutrient availability, moisture, pH, temperature, plant-root activity and land-use practices.

Table.1 Sampling sites included in the study.

Site code	Sampling location
S1	Tomato field
S2	College ground
S3	Crop cafeteria
S4	Mulberry field
S5	Orchard
S6	Sapota field
S7	DBCA wetland
S8	Kilvenbakkam
S9	Takkolam
S10	Paddy field

Table.2 Population density of bacteria in soil samples from the ten sampling sites. (10^{-5} CFU g^{-1} soil)

Site	Location	M1	M2	M3	Mean*
S1	Tomato field	8.5	10.5	9.7	9.53cdefg
S2	College ground	9.1	10.7	9.4	9.71cde
S3	Crop cafeteria	7.4	6.9	8.1	7.45hi
S4	Mulberry field	5.8	7.2	6.7	6.54ij
S5	Orchard	9.5	10.1	11.0	10.18c
S6	Sapota field	14.2	16.0	14.8	14.98a
S7	DBCA wetland	12.8	15.4	13.6	13.89ab
S8	Kilvenbakkam	7.2	8.1	10.2	8.41cdefgh
S9	Takkolam	9.0	9.8	11.2	9.96cd
S10	Paddy field	8.8	9.6	10.4	9.58cdef

Table.3 Population density of fungi in soil samples from the ten sampling sites.

Site	Location	M1	M2	M3	Mean*
S1	Tomato field	20.5	22.8	21.7	21.65ab
S2	College ground	10.2	12.8	16.4	12.89cdefgh
S3	Crop cafeteria	17.8	20.5	19.5	19.23abc
S4	Mulberry field	7.6	11.9	14.3	10.89defghi
S5	Orchard	11.8	13.6	15.2	13.46cdefg
S6	Sapota field	6.9	18.5	6.2	9.25fghij
S7	DBCA wetland	23.5	25.48	24.7	24.55a
S8	Kilvenbakkam	15.8	18.4	17.3	17.13abcd
S9	Takkolam	12.0	14.5	16.0	14.07bcdef
S10	Paddy field	14.5	16.2	15.8	15.48bcde

Table.4 Population density of actinobacteria in soil samples from the ten sampling sites.

Site	Location	M1	M2	M3	Mean*
S1	Tomato field	12.4	13.9	13.5	13.25bcdefg
S2	College ground	13.7	15.0	14.5	14.39abcdef
S3	Crop cafeteria	15.1	16.7	16.0	15.92a
S4	Mulberry field	14.0	15.6	15.1	14.88abcd
S5	Orchard	14.3	15.8	15.2	15.09abc
S6	Sapota field	5.7	7.5	6.3	6.46ij
S7	DBCA wetland	6.1	7.9	6.9	6.93i
S8	Kilvenbakkam	9.2	11.4	10.5	10.33h
S9	Takkolam	14.5	16.1	15.4	15.32ab
S10	Paddy field	13.8	15.3	14.8	14.62abcde

Table.5 Comparative microbial profile of selected sites.

Site	Bacteria	Fungi	Actinobacteria	Overall profile
S6 – Sapota field	Highest (14.98)	Lowest (9.25)	Lowest (6.46)	Bacteria-dominated
S7 – DBCA wetland	High (13.89)	Highest (24.55)	Low (6.93)	Fungal/bacterial-dominated
S3 – Crop cafeteria	Low (7.45)	High (19.23)	Highest (15.92)	Fungal/actinobacterial

Sample Collection from Takkolam

Sample Collection from DBCA

Sample Collection from nursery

Soil Sample Storage

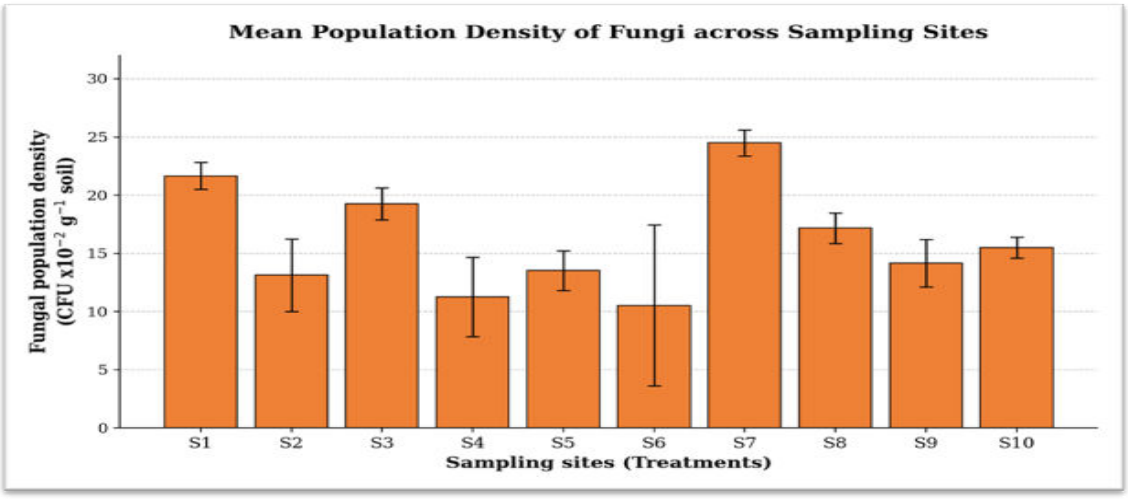
Labeling of Soil Samples

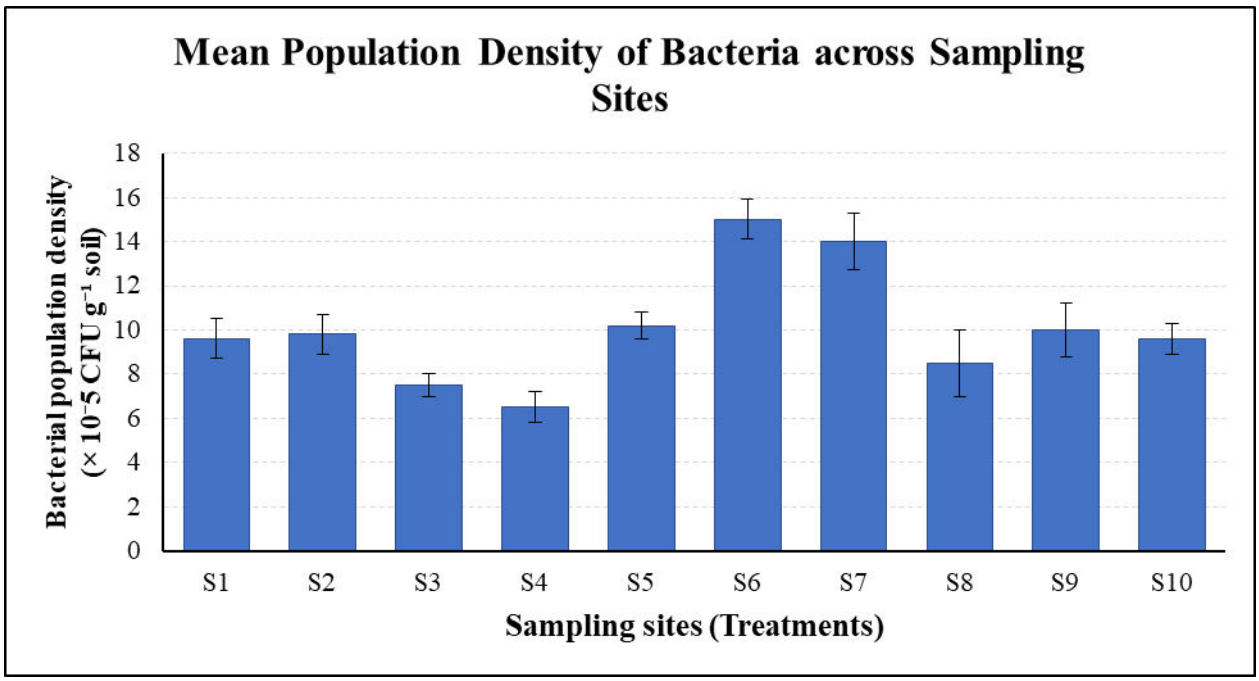
Serial Dilution of Soil Sample

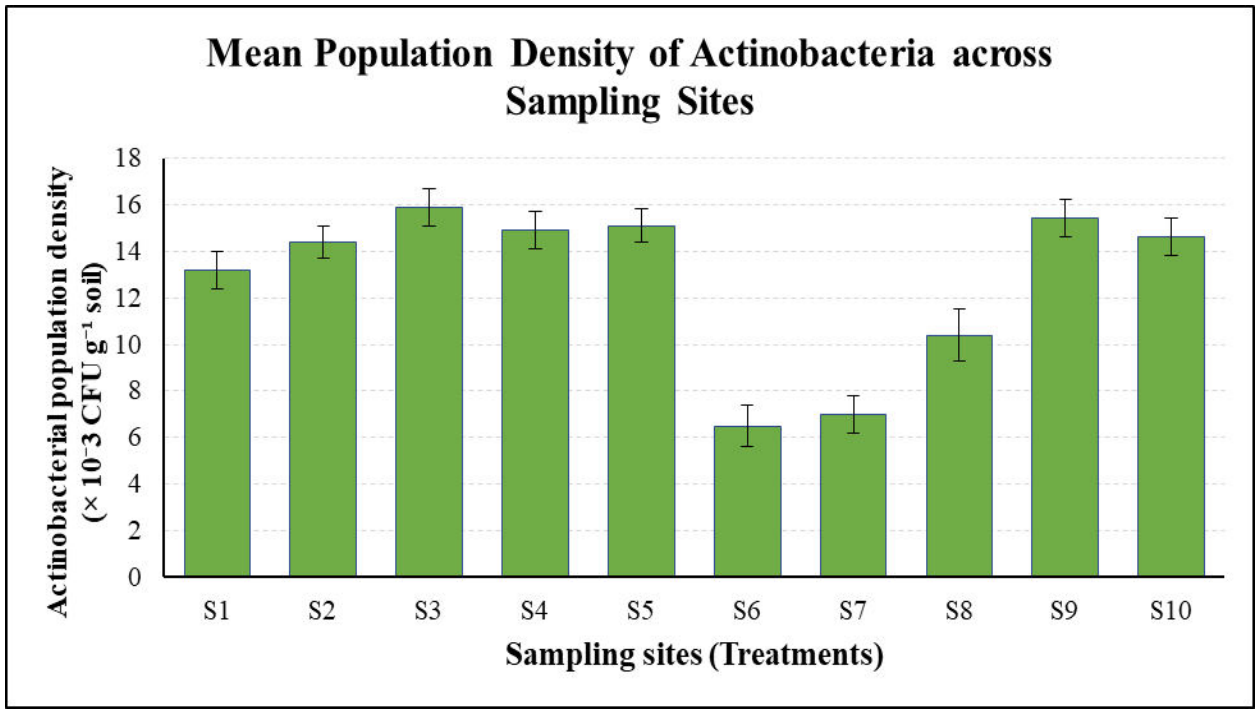
	
	
Soil Sample Storage	**Weighing of Media Components**
	
Microscopic Observation of Bacteria	**Microscopic Observation of Fungi**

Identification of Microorganisms (*Aspergillus niger*)

Counting of Microorganism Colonies







Prabha *et al.,* (2025) similarly emphasized the importance of environmental gradients in shaping microbial abundance and community structure.

The bacterial population was highest in the sapota field, followed by the DBCA wetland, while the mulberry field had the lowest population. The relatively high bacterial population in the sapota field may be compatible with sustained root-derived inputs and organic substrates associated with perennial vegetation. Moisture availability in the wetland may also provide favourable conditions for bacterial growth. However, because soil pH, organic carbon, moisture and nutrient concentrations were not directly measured in the present investigation, these factors should be regarded as plausible explanations rather than demonstrated causes.

Fungal populations showed a contrasting pattern. The DBCA wetland recorded the highest fungal population, whereas the sapota field recorded the lowest. Moisture and organic substrate availability may contribute to this distribution because many soil fungi function as decomposers of complex organic materials (Khan *et al.,* 2023). The relatively large variation among fungal replicates in the sapota field (6.9, 18.5 and 6.2 × 10^{-2} CFU g^{-1} soil) indicates considerable within-site heterogeneity and should be considered when interpreting the site mean.

Actinobacteria exhibited the strongest statistical site effect among the three microbial groups. The crop cafeteria recorded the highest population, whereas the sapota field had the lowest. The comparatively low actinobacterial population in the DBCA wetland may be compatible with reduced oxygen diffusion under saturated conditions because many commonly cultured soil actinobacteria are predominantly aerobic.

Nevertheless, waterlogging was not directly measured, so this remains a possible environmental explanation rather than a demonstrated mechanism. The contrasting microbial profiles indicate that bacteria, fungi and actinobacteria did not respond uniformly to the land-use conditions represented by the sampling sites. The sapota field was characterized by a bacterial-dominated profile, the DBCA wetland by comparatively greater fungal abundance, and the crop cafeteria by high actinobacterial abundance. These differences support the view that soil microbial groups occupy different ecological niches and respond differently to local environmental conditions.

From a bioremediation perspective, the recovery of substantial culturable microbial populations provides a preliminary resource for future functional screening (Godheja *et al.,* 2016). Bacterial isolates from sites with high bacterial abundance could be evaluated for transformation of selected pesticides, hydrocarbons or other target compounds (Jha *et al.,* 2018). Fungal isolates from the DBCA wetland could be screened for extracellular enzyme production and transformation of structurally complex organic pollutants (Kumar *et al.,* 2021), while actinobacterial isolates from the crop cafeteria and other high-abundance sites may be evaluated for transformation of recalcitrant organic substrates. A consortium approach may eventually be useful because different microbial groups can contribute complementary metabolic functions (Sharma *et al.,* 2024).

Importantly, microbial abundance alone does not establish xenobiotic degradation. The present investigation did not quantify degradation of a defined xenobiotic compound, measure residual pollutant concentration, identify degradation metabolites or characterize degradation enzymes. Therefore, the findings should be interpreted as a microbial population and screening study rather than as direct evidence of xenobiotic biodegradation. Future investigations should combine molecular identification of promising isolates with compound-specific degradation assays, residual chemical analysis, metabolite characterization, enzyme assays and controlled soil microcosms (Lennartz *et al.,* 2024). Such approaches are consistent with the need for functional characterization of xenobiotic-degrading microorganisms and microbial communities (Mishra *et al.,* 2021).

The principal methodological limitation is the culture-dependent nature of the enumeration. Only microorganisms capable of growing under the selected media and incubation conditions were recovered, and therefore the reported CFU values do not represent total microbial abundance. In addition, physicochemical properties of the sampling soils were not measured, limiting causal interpretation of the observed site differences. The morphological identification of *Aspergillus niger* should also be confirmed by molecular methods such as ITS sequencing if species-level identification is required.

In conclusion, the present investigation demonstrated significant variation in culturable bacterial, fungal and actinobacterial populations across ten land-use sites in and around DBCA, Sagayathottam, Ranipet District. The highest bacterial population was recorded in the sapota field, the highest fungal population in the DBCA wetland, and the highest actinobacterial population in the crop cafeteria. These contrasting patterns demonstrate substantial spatial variation in culturable soil microbial communities.

The recovered microbial populations provide a preliminary basis for selecting indigenous isolates for future xenobiotic-degradation and bioremediation screening. However, the study did not demonstrate degradation of any specific xenobiotic compound. Compound-specific degradation assays, chemical residue analysis, molecular identification and controlled experiments are required before individual isolates can be conclusively described as xenobiotic degraders.

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Author Contributions

D. R. Sudha: Investigation, formal analysis, writing—original draft. R. Beulah Bhakiya Sherlin: Validation, methodology, writing—reviewing. M. Hariharan:—Formal analysis, writing—review and editing. J. Hillary Karen: Investigation, writing—reviewing. A. R. Joel Johnson: Resources, investigation writing—reviewing. R. Karthick: Validation, formal analysis, writing—reviewing. S. Kumar: Conceptualization, methodology, data curation, supervision, writing—reviewing the final version of the manuscript. A. Maranvazhuthi: Investigation, formal analysis, writing—original draft.

Data Availability

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethical Approval Not applicable.

Consent to Participate Not applicable.

Consent to Publish Not applicable.

Conflict of Interest The authors declare no competing interests.

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