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Microbial population and diversity in earthworm midden of *Ocnerodrilus occidentalis* Eisen

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Abstract- The effect of earthworm (*Ocnerodrilus occidentalis* Eisen) middens on microbial population density, biomass and bacterial diversity has been studied in laboratory. Initially bacterial population was high in midden (57.2 ± 2.804 X10^9) than soil (45.2 ± 0964 X 10^9) there after a sharp decline in bacterial population was observed both in soil and earthworm midden. The bacterial species reported in the midden of *O. occidentalis* belong to the genus *Aeromonas, Bacillus, Pseudomonas, Sphingomonas, Kocuria, Acinetobacter* and *Methylobacterium*. The objective of the present study was to analyse the state of art on the microbial population and bacterial diversity in the midden of *O. occidentalis*.

Key words: Midden, *O. occidentalis*, Bacterial diversity, *Bacillus*

INTRODUCTION

Earthworms are considered to be important ecosystem engineers in terrestrial ecosystem.[1] Earthworms are frequently associated with their ability to mix the soil, increase water infiltration rate, improve soil aeration, nutrient cycling, build soil structure and increase the biological activity of the soil.[2-8] The soil is used as a medium for plant growth whose productivity and stability greatly depends on the balance between living and non-living components. Crop plants stores the essential nutrients and energy from sun required for proper growth and are recycled in to the soil by the help of micro and macro-organisms through decomposition process.[9] Qualitative and quantitative microbial activities are the key factors for productivity and sustainability of soils health for maintenance of crop production.[10-12] Mechanical mixing of the mineral particles and organic matter in the digestive system of earthworms may result in increasing or decreasing the activity and number of beneficial or pathogenic microorganisms.[13]

Earthworm casts are hot spots of nutrient dynamism and microbial activity and may be used as model for studying the influence of earthworm soil microbial communities by making some changes in the patch structure of the microbial environment.[14] We studied the structure and activity of the bacterial communities in the soil and surrounding soil and measure implement in the agricultural field. The present study reports for the first time the microbial population from the midden of worm *Ocnerodrilus occidentalis.*

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MATERIAL & METHODS

Soil sample collection

Sampling was done to collect the earthworm *Ocnerodrilus occidentalis* Eisen from different agroecosystem sites in Ranchi, located between 21°58'N - 25°19, NL and 83°20'E- 88°4'EL and at a height of 629m above mean sea level (MSL) and study was carried out in

laboratory by culturing the earthworms in plastic container under oxygenated and moist condition. The middens were collected from the plastic container and used for microbial study.

Bacterial culture and isolation

The bacterial population in midden and soil was estimated by Dilution plate method.[15] The bacteria were isolated from soil samples by taking 1g of sample which was diluted with 9 ml of sterilized deionized water till 10^{-7} dilution. 1 mL inoculums of the primary suspension were taken for bacteria culture in a petriplate (diameter= 100mm) containing CzapekDox agar media and were inoculated at 37°C for 48h.[16] After that colony count were continued at every interval of 7 days till 42nd day.

Isolation of DNA and genomic analysis

Identification of the bacteria from midden and soil were done by isolation of DNA and genomic analysis. For the total DNA isolation from the pure culture, bacterial cells were washed with TESS buffer [10 mMtris/HCl, 1mM Na_2EDTA, 0.1 M NaCl and 0.1% Sarkosyl (N- lauroyl sarcosine)] and resuspended in TE buffer (10 mMTris/HCl, 1 mM Na_2EDTA). 50 mg lysozyme mL-1 and 0.1% SDS were used to lyse the cells. The subsequent phenol / chloroform extractions and ethanol precipitation were carried out as described by Sambrook *et al.* (1989)[17]. The quantity of the DNA was checked by running on 1.2% agarose gel. A single band of DNA with high molecular weight has been observed. The extracted genomic DNA of both isolates was used as template DNA for amplification of the 16S rDNA gene. PCR amplification of the Fragment of 16S rDNA gene was DONE. The purification of PCR amplicon was done to remove contaminants. Forward and reverse DNA sequencing was carried out by using BDT v 3.1 cycle sequencing kit on ABI 3730 X 1 genetic analyzer and consensus sequence was generated by Aligner software. The 16S rDNA gene sequence was used to carry out BLAST with nr database of NCBI gene bank database.[18,19]

RESULTS & DISCUSSION

The bacterial population in soil and in midden during the study period has been presented in Table 1. The, bacterial population in soil gradually decreased from $45.2\pm0.964X10^9$ to $4.8\pm0.665X10^9$ on 42 days of observation. On 7th day of observation bacterial population in midden was $47.9\pm0.568X10^9$ which gradually decreased to $11.5\pm0.568X10^9$. The bacterial population in midden was always higher than soil and is in conformity with the findings of Kumari *et al.*, (2010)[20]. The population showed

Table 1: Bacterial population in soil and midden

Days of observation	Bacterial population in soil (M±SD)	Bacterial population in midden (M±SD)	% Change
0	$45.2 \pm0.964\ X10^{9*}$	$57.2 \pm2.804X10^{9*}$	+26.54%
7	$35.1 \pm1.053X10^{9**}$ (-22.34)	$47.9 \pm0.568\ X10^{9**}$ (-16.25)	+36.46%
14	$19.1 \pm2.003\ X10^{9**}$ (-57.74)	$33.9 \pm1.738\ X10^{9**}$ (-40.73)	+77.48%
21	$14.6 \pm1.588X10^{9**}$ (-67.69)	$27.6 \pm0.450\ X10^{9**}$ (-51.74)	+89.04%
28	$12.3 \pm0.611\ X10^{9NS}$ (-72.78)	$25.2 \pm0.617X10^{9NS}$ (-55.94)	+104.87%
35	$6.7 \pm1.450\ X10^{9**}$ (-85.17)	$16.7 \pm0.929\ X10^{9**}$ (-70.80)	+149.25%
42	$4.8 \pm0.665\ X10^{9**}$ (-89.38)	$11.5 \pm0.568\ X10^{9**}$ (-79.89)	+139.58%

Values in parenthesis are percentage increase (+) or decrease (-) over initial value; Significant level *=pd"0.01; **=pd"0.001; NS=non-significant; n=3

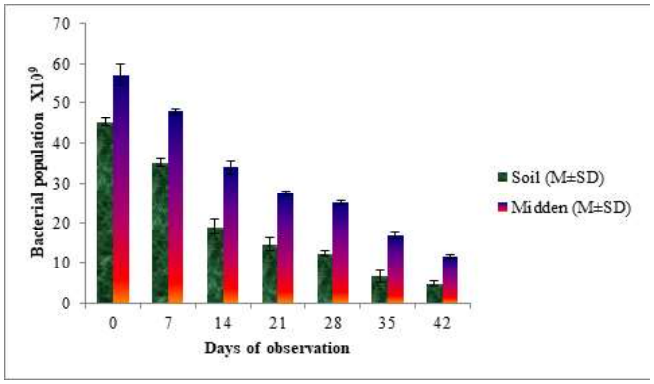

Figure 1- Bacterial population of soil and midden

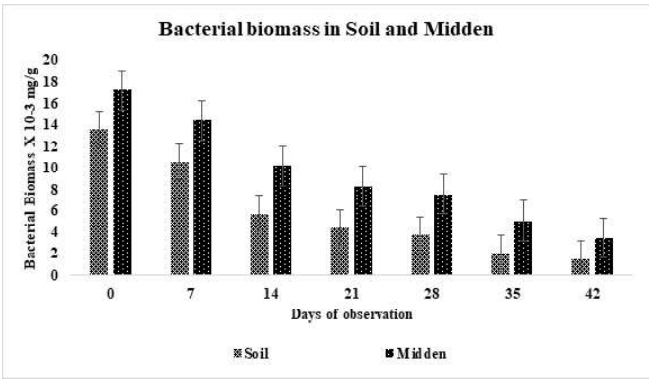

Figure 2- Bacterial Biomass of soil and midden.

significant increase from soil to midden ($p<0.001$) (Fig. 1). The maximum % change (149.25%) between bacterial population of soil and midden was observed in 35th day of observation. Initially percentage increase from bacterial population of soil to midden was found to be 26.54% which was gradually increased to 149.25%. The percentage decrease in bacterial population of soil over initial population was recorded as 22.34%, 57.74% and 67.69% on 7th, 14th and 21st day while more pronounced as 72.78%, 85.17% and 89.38% on 28th, 35th and 42nd day respectively (Table 1). The percentage decrease over initial bacterial population of midden was observed as 16.25% on 7th day which gradually increased due to aging of midden.

The wet weight (mg/g soil) of bacterial population in soil decreased by 22.34% (67.8±2.625 X 10^{-3} to 52.65±1.580 X 10^{-3}) on 7th day. On 14th and 21st day the decrease was in order of 57.74% and 67.69%. After 21st day, wet weight decreased was more pronounced 89.38% on 42nd day (Table 2). A similar trend of fall in biomass (mg/g soil) was observed. The initial biomass was observed as 13.56±0.525 X 10^{-3} which was gradually decreased to 1.44±0.199 X 10^{-3} on 42nd day (Fig 2). The initial wet weight (mg/g midden) of bacterial population in midden was 85.80±4.206 X 10^{-3} which decreased 16.25% on 7th day and 51.74% on 21st day (41.40±0.676 X 10^{-3}). A similar trend of fall in biomass was also observed in midden. The maximum biomass 17.16±0.841 X 10^{-3} was observed in first day and thereafter declined to 3.45±0.170 X 10^{-3} on last day of observation (Fig. 2).

Table 2: Wet weight of bacterial population of soil and midden

Days of observation	Soil (M±SD)	Midden (M±SD)	% Change
0	67.8±2.625 X10^{-3}	85.80±4.206 X10^{-3}	+26.54%
7	52.65±1.580X10^{-3} (-22.34)	71.85±0.852X10^{-3} (16.25)	+36.46%
14	28.65±3.004 X10^{-3} (-57.74)	50.85±2.608X10^{-3} (40.73)	+77.48%
21	21.90±2.382 X10^{-3} (-67.69)	41.40±0.676X10^{-3} (51.74)	+89.04%
28	18.45±0.916 X10^{-3} (-72.78)	37.8±0.916 X10^{-3} (-55.94)	+104.87%
35	10.05±2.175 X10^{-3} (-85.17)	25.05±1.393X10^{-3} (-70.80)	+149.25%
42	7.20±0.998 X10^{-3} (-89.38)	17.25±0.852X10^{-3} (-79.89)	+139.58%

Values in parenthesis are percentage increase (+) or decrease (-) over initial value

The literature review reveals some controversy on the role of earthworms on the size of the soil microbial biomass. Several studies have shown that earthworms reduce microbial biomass, mainly by consumption when the soil travels through the alimentary tract of earthworm.[21-28] In contrast, other studies have found earthworm induced increases in microbial biomass.[20,29-33] Devliegher and Verstraete (1995)[23] suggested that the net effect of earthworm on microbial biomass is a product of reductions in biomass during gut passage and stimulation due to mixing of organic matter into the soil profile. Brown *et al.*, (2000)[34] emphasized the importance of temporal and spatial scale when evaluating the effects of earthworms on the soil profile, suggesting the different behaviour of fresh earthworm casts than aged casts.

The main nutritional source of organic matter for earthworms is the plant detritus however a few microorganisms like have been sometimes found to be part of the diet of these worms.[35-37] This relationship between microorganisms and worms isn`t essentially restricted to a predatory process, as it has been reported that earthworms' digestive enzymes do not have significant impact on some microorganisms.[38]

On the basis of genomic analysis, identified 11 species of bacteria from the genus *Aeromonas, Bacillus, Sphingomonas, Acinetobacter, Methylobacterium, Brevundimonas, Pseudomonas* were found in the soil while one more *Kocuria* was found in the midden of *Ocnerodrilus occidentalis*. Seven species of bacteria of the genus *Bacillus* were identified (*B. insolitus, B. megaterium, B. brevis, B. pasteurii, B. sphaeriaus, B. thuringiensis* and *B. pabuli*) within the intestine of *Onychochaeta boincana.*[39]

An observation was made for the midden of the species *Ocnerodrilus occidentalis*, there was an increase in 572 colonies, further divided into 7 groups: *Aeromonas* 17%, *Bacillus* 53%, *Pseudomonas* 10%, *Sphingomonas* 4%, *Kocuria* 2%, *Acinetobacter* 11%, *Methylobacterium* 3% (Table 3). The genus *Bacillus* was dominant group found in the midden of *Ocnerodrilus occidentalis*. Hyun Jung *et al.* (2004)[37] reported that the genus *Bacillus* was

dominant group found in the intestine of the earthworm. Some species include *Bacillus cereus, B. subtilis, B. thuringiensis, Pseudomonas aerufasciens, P. putida*. In comparison to soil the genus *Bacillus, Aeromonas* were increased in the midden of *Ocnerodrilus occidentalis*.

Table 3: Percentage occurrence of bacteria isolated from soil and earthworm midden.

Identified species	Family	Soil	Midden
Aeromonas punctata strain JM10	Aeromonadaceae	16.5 %	17.4 %
Bacillus cereusprobio 32	Bacillaceae	26.7 %	31.4 %
Kocuria HO-9042	Micrococcaceae	-	1.7 %
Sphingomonas	Sphingomonadaceae	9.9 %	4.3 %
Pseudomonas	Pseudomonadaceae	9.5 %	9.9 %
Acinetobacter	Moraxellaceae	10.8 %	10.4 %
Brevundimonas	Caulobacteraceae	6.1 %	-
Methylobacterium	Methylobacter	-	2.6 %
Bacillus MBL13	Bacillaceae	7.9 %	7.8 %
Bacillus BFF-3	Bacillaceae	8.8 %	9.6 %
Bacillus HBUM 84231	Bacillaceae	2.8 %	4.3 %

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