Research Journal of Agricultural Sciences
An International Journal

Res. Jr. of Agril. Sci. **12**(2): 478–482

P- ISSN: 0976-1675 | **E**- ISSN: 2249-4538

www.rjas.org

Research Paper

Bio-potent Bacterial Population in the Vermicompost of *Eudrilus eugeniae* in different Concentrations of Plant Waste with Cow-dung Mixture

B. Prakash[1], S. Durai Raj[2] and T. Murugan*[3]

Received: 09 Jan 2021 | Revised accepted: 06 Mar 2021 | Published online: 18 Mar 2021

A B S T R A C T

The microbial population present in vermicompost play an important role in increasing the productivity of crop as well as maintain the structural stability of the soil. This study was carried out to identify the total viable bacterial population in vermicompost using cow-dung and plant waste vermicompost by the worm *Eudrilus eugeniae*. In this study, the total viable count of bacteria was enumerated in both worn unworked substrate (before worms introduced) and *E. eugeniae* worked compost. Compare to worm unworked substrate; the vermicompost consisted of higher population of bacterial cultures. In this investigation, a maximum of 48.3 ± 1.5 CFU x 10^5 g^{-1} was recorded in Exp. 3 of processed vermicompost. Also, higher population dominant bacterial cultures recovered in processed vermicompost than unworked substrate. A total of six dominant bacterial strains were probably identified as *Micrococcus luteus, Bacillus cereus, Bacillus subtilis, Azotobacter sp., Enterobacter sp.* and *Pseudomonas aeruginosa.* The bacterial population was found to be significantly greater in the *E. eugeniae* worked vermicompost.

***Key words*:** Vermicompost, Cow-dung, *Eudrilus eugeniae,* Total viable count, Vermitechnology

Vermicompost is a new and promising choice for sustainable agriculture that is commonly used to grow various agricultural and horticultural plants [1]. This process consumes various types of agricultural wastes such as agricultural residues, cattle dung, sewage sludge and many organic industrial residues [2). Earthworms are being used to treat a wide variety of organic wastes found in the land. The application of vermicompost in agricultural fields may stimulate the load of soil microorganisms and promotes plant growth by providing various micro and macro nutrients [3-4].

Microorganisms are essential part of biodiversity and play a significant role in the structuring and functioning of the ecosystem in the environment [5]. Earthworms and symbiotic gut microflora secreted mucus and water to increase their degradation rate of ingested organic matter and the release of assimilable metabolites. Thus, the microorganisms and earthworms act symbiotically to accelerate and enhance the decomposition of organic matter and as a consequence, mineralization and humification takes place resulting in the availability of nutrients for plants [6-9].

The beneficial microorganisms found in the compost promote plant growth by mutualistic or symbiotic relationships by mineralization of plant nutrients bound in organic matter, by fixing nitrogen and by controlling root diseases. The activities of the earthworms in the compost have been shown to promote the dispersal through soil of a variety of beneficial microorganisms including *Azotobacter*, *Pseudomonas*, *Rhizobi*a and mycorrhizal fungi [10]. This present work is aimed to enumerate total microbial population and identification of the dominant bacterial genera in the vermicompost using the worm *Eudrilus eugeniae.*

*T. Murugan
tmurugamb@gmail.com

[1-2]P. G. and Research Department of Zoology, Arignar Anna Government Arts College, Cheyyar - 604 407, Tamil Nadu

[3]Department of Microbiology, Sri Sankara Arts and Science College, Enathur, Kanchipuram - 631 561, Tamil Nadu, India

MATERIALS AND METHODS

Plant waste and pre-composting

Fruit peel waste of *Ceiba pentandra* was collected from in around of Cheyyar, Thiruvannmalai District, Tamil Nadu (12.67° N, 79.55° E). The collected peel wastes were dried under shade condition and manually ground with the aid of electric mixer. The powdered plant waste was mixed with dried cow-dung powder for the preparation of vermibed. The mixture was kept in plastic container for a period of 21 days for initial composting and initiation of microbial degradation and softening of waste mixture. During this period, the waste mixture in different bedding was turned out periodically (after 5 days) for proper aeration and remove odour from decomposing wastes [11].

Earthworm

The earthworm *Eudrilus eugeniae* was collected from Vedhapuri Agricultural form in Chithathur, Thiruvannmalai District, Tamil Nadu. The collected worms were immediately transported into the laboratory and kept in earthen pot (5 L vol.), which was partially filled with a mixture of loamy and humus soil supplemented with cow-dung, dry leaves and some

vegetable wastes [12-13]. After 21 days earthworm of similar sizes was carefully selected from the earthen pots and used for further studies.

Vermicomposting

Composting process was carried out with six replicates in a series of 5L capacity pots. Pots were filled with dry humus soil, powdered form of plant waste and dry cow-dung powder in different proportions and coded as experimental setup 1 to 6 and one setup without plant waste was used as control. In each experimental setup 10 earthworms were added for composting. The process of vermicomposting was carried out for a period of 40 days. The temperature and moisture content were maintained by sprinkling adequate quantity of water at frequent intervals [14].

Experimental setup

Control: Soil (2 Kg) + Cow-dung (25 g)
Exp. 1: Soil (2 Kg) + Cow-dung (25 g) + Plant waste (50 g)
Exp. 2: Soil (2 Kg) + Cow-dung (25 g) + Plant waste (100 g)
Exp. 3: Soil (2 Kg) + Cow-dung (25 g) + Plant waste (150 g)
Exp. 4: Soil (2 Kg) + Cow-dung (50 g) + Plant waste (25 g)
Exp. 5: Soil (2 Kg) + Cow-dung (50 g) + Plant waste (50 g)
Exp. 6: Soil (2 Kg) + Cow-dung (50 g) + Plant waste (100 g)

Determination of microbial population

In the present study, the compost processed by *E. eugeniae* using *C. pentandra* fruit peel waste was investigated for total viable bacterial count by serial dilution plate method [15]. The soil from each experimental setup was serially diluted by mixing 1 g of soil into 100 ml of sterile saline into a 250 ml of conical flask. The suspension was kept in a rotary shaker for 30 min. A series of test tubes with 9 ml of saline was taken in a test tube rack and marked as respective dilutions. 1 ml of the suspension was transferred into 9 ml of saline into a test tube. The tube was mixed thoroughly and then 1 ml was transferred into another tube. Likewise, serial dilution was made up to 10-6 dilutions. For the isolation of bacteria, 0.1 ml from each dilution such as 10-3, 10-4 and 10-5 were taken with help of clean micropipette and separately inoculated into a sterile Nutrient agar (Himedia, Mumbai) plate. The inoculums were evenly spread with the aid of sterile glass L-rod and the plates were incubated at 37°C for 24-48 hours. After incubation, the plates were observed for total bacterial populations.

Selection of dominant pure cultures

After counting of total bacterial population, the plates showing morphologically distinct colonies were observed. The selected bacterial colonies were made sub-cultured into a sterile nutrient agar plates for characterization [16].

Characterization and identification of bacterial cultures

The isolated dominant bacterial was conventionally identified by studying cultural, morphological and biochemical characteristics. The following tests were performed for the identification of bacteria viz. colony morphology, Gram staining, catalase test, oxidase test, endospore staining, indole test, methyl red test, voges proskauer test, citrate test, urease test, triple sugar iron test, gelatin hydrolysis test, starch hydrolysis test, casein hydrolysis test and sugar fermentation test with various sugars such as glucose, fructose, sucrose, xylose, mannitol, lactose and maltose. The test results were compared with Bergey's manual of determinative bacteriology and the organisms were identified [17].

RESULTS AND DISCUSSION

Earthworm and plant waste

A wide variety of earthworm species like *Drawida willis, Eudrilus eugeniae, Eisenia andrei, Eisenia fetida, Lampito mauritii, Lampito rubellus, Megascolex mauritii* and *Perionnyx excavates* are utilized for the conversion of organic wastes into vermicompost [18-19]. Among these, the commonly available two species such as *Eudrilus eugeniae* and *Eisenia foetida* are widely used varieties in India [20]. Generally, vermicomposting by the earthworm *Eudrilus eugeniae* can be processed from various types of raw materials such as agricultural residues, sugar cane thrash, cattle manure, gaur gum, municipal solid waste, municipal, agricultural and mixed solid waste, onion waste, press mud, wooden or plastic waste and vegetable waste and floral waste mixture [21-22]. In the present investigation, a mixture of cow dung and fruit peel waste of *Ceiba pentandra* with different proportions were used as raw materials for vermicomposting.

Vermicomposting

Cow-dung is a familiar raw material for the production of vermicompost used in various proportions. In the present study, vermicompost was processed with the addition of *Ceiba pentandra* fruit peel waste in different proportions. In this investigation, it was observed that the degradation or conversion rate of the fruit peel waste into compost was high and also the yield of the vermicompost was high. Cow-dung takes place a most important place in earthworm diet and most kinds of animal dung are highly used for the preparation of vermicompost because they containing many nutritious food sources for earthworms [23]. The cow-dung is able to increase the stability of the material to be converted into vermicompost as a feed for both microorganisms and earthworms [24]. It significantly minimizes the mortality rate and increases the length of the worms; also increase the worm's populations in the compost [25-27]. As well, the addition of *Ceiba pentandra* fruit peel waste as a raw waste material considerably increases the degree of the production of vermicompost.

Determination of microbial population

The total viable count of bacteria was determined in initial compost before worms were introduced and vermicompost after 40 days of incubation. In the present investigation, the viable counts of bacterial colonies ($CFUx10^5g^{-1}$) in initial substrates were ranged from 41.7±2.1 to 43.7±2.1 and 40 days vermicomposts were ranged from 44.3±1.2 to 48.3±1.5. Also, maximum number bacterial count was recorded in the vermicompost taken from Exp. 3 (48.3±1.5) and Exp. 4 (48.7±1.4) and minimum from control (44.3±1.2). In this study, it was observed that, the viable bacterial count was increased in the vermicompost (40 days compost) than it was observed in initial substrate (before worms introduced) (Table 1). These results indicated that, the introduction of earthworm into the organic wastes may stimulate the quantity of viable microorganisms. These results are in conformity with the result of earlier works like [28] who had reported higher counts of bacteria when the *E. eugeniae* worked organic waste mixed with soil. An increase of bacterial population in *E. eugeniae* worked vermicompost has reported by [29].

Table 1 Total viable bacterial count of vermicompost by *E. eugeniae*

Experimental setup	CFU × 105g^{-1}	
	Before worms introduced	After 40 days of vermicompost
Exp. 1	41.7 ± 2.1	45.7 ± 2.9
Exp. 2	42.7 ± 1.2	46.7 ± 1.2
Exp. 3	43.7 ± 2.1	48.3 ± 1.5
Exp. 4	43.3 ± 1.5	48.7 ± 1.4
Control	41.3 ± 3.1	44.3 ± 1.2

Selection of dominant pure cultures

The dominant bacterial colonies were recovered in both initial substrate and processed vermicompost. In this investigation, the initial substrate showed two dominant bacterial colonies in Exp. 1, 2, 4 and control setup and three in Exp. 3. Likewise, in *E. eugeniae* processed vermicompost showed three dominant colonies in control; four dominant colonies in Exp. 1 and 2; and each 5 colonies from Exp. 3 and 4 (Table 2). Also, a maximum number of dominant bacterial colonies were recorded in Experimental setup 3 and 4 which containing high quantities of *C. pentandra* fruit peel waste. These results proved that; the organic compost processed by *E. eugeniae* may increase the quantity as well as the species of bacteria, especially plant growth promoting microorganisms. The increase of microbial population may be caused by congenial condition for the growth of microbes within the worm digestive tract and by the ingestion of nutrient rich organic wastes which provide energy and also act as a substrate for the growth of microorganisms as reported by [30].

Table 2 Morphologically distinct dominant bacterial colonies of vermicompost by *E. eugeniae*

Experimental setup	Before worms introduced	After 40 days of vermicompost
Exp. 1	2	4
Exp. 2	2	4
Exp. 3	3	5
Exp. 4	2	5
Control	2	3

Characterization and identification of bacterial cultures

The dominant bacterial colonies with similar colony morphologies were selected from all the experimental setup of both initial substrate and processed vermicompost. In this study, six dominant bacterial strains (coded as DS1- DS6) were recorded from the entire samples. The bacterial strains were identified by studying cultural, morphological and biochemical characteristics. Based on the above characteristics, the dominant bacterial strains DS1, DS2, DS3, DS4, DS5 and DS6 were probably identified as *Micrococcus luteus*, *Bacillus cereus*, *Bacillus subtilis*, *Azotobacter sp.*, *Enterobacter sp.* and *Pseudomonas aeruginosa* (Table 3).

Table 3 Characterization and identification of bacterial cultures isolated from the vermicompost by *E. eugeniae*

S. No.	Test parameters	DS1	DS2	DS3	DS4	DS5	DS6
1	Colony morphology	Yellow, circular, raised	White, raised, lobate	Dirty white, raised, lobate	White, raised, lobate	Dirty white, raised	Yellowish green, mucoid
2	Gram staining	G +ve	G +ve	G +ve	G +ve	G -ve	G -ve
3	Cell morphology	Cocci	Rod	Rod	Irregular rod	Rod	Rod
4	Catalase test	+	+	+	+	+	+
5	Oxidase test	+	+	-	+	-	+
6	Spore	-	+	+	-	-	-
7	Motility	-	+	+	+	+	+
8	Indole test	-	-	-	+	-	-
9	MR test	-	-	-	-	-	-
10	VP test	+	+	+	-	+	+
11	Citrate test	-	+	+	+	+	+
12	Urease test	-	+	+	+	-	
13	TSI test	A/A	ALK/A	A/A	A/A	ALK/A	ALK/ALK
14	Gelatin hydrolysis	+	+	+	-	-	+
15	Glucose	-	+	+	+	+	+
16	Fructose	+	+	+	+	+	+
17	Sucrose	-	+	+	+	+	-
18	Lactose	+	-	+	+	-	-
19	Maltose	-	+	+	-	+	-
Probable Identity of the organism		*Micrococcus luteus*	*Bacillus cereus*	*Bacillus subtilis*	*Azotobacter sp.*	*Enterobacter sp.*	*Pseudomonas aeruginosa*

Among the identified dominant bacterial strains, *Micrococcus luteus* was isolated in all the experimental setup except Exp. 4 of initial substrate; *Bacillus cereus* was recorded in all samples except Exp. 2 of initial substrate; and the strain *Bacillus subtilis* was isolated only in entire vermicompost sample but not in any initial substrate (before worms introduced). Similarly, *Azotobacter sp.* recovered from all samples except control of initial substrate; *Enterobacter sp.* and *Pseudomonas aeruginosa* were identified from the entire samples of both initial substrate and processed vermicompost. In this study, it was observed that, *Bacillus subtilis* was only isolated from the samples of vermicompost by *E. eugeniae.* This work was supported by the work done by [5], in their research, the vermicompost of *E. eugeniae* showed high quantity of viable microbial populations. Also, the bacterial strain such as *Enterobacter acrogens, Enterococcus faecivm, Citrobactor diversus, Escherichia coli, Pseudomonas aeruginosa and Proteus vulgaris* were identified from worm unworked natural compost and strains such as *Bacillus subtilis, Bacillus cereus, Enterobacter aerogenes, Enterococcus faecium, Escherichia coli, Klebsiella pneumoniae, Proteus vulgaris, Pseudomonas aeruginosa* and *Morganella morganii* were identified from *E. eugeniae* processed compost [31-32].

CONCLUSIONS

Vermicomposting is a technology that focuses on the conservation of various waste resources and their sustainable utilization. It can also be used for the treatment of different organic wastes like plants, cardboard, paper, manures, food and bio-solids etc. Vermicompost has higher economic value compared to compost derived from traditional methods. The study supports the presence of a group of bacterial strains in the vermicompost produced from plant wastes and cow-dung. These finding states that, all these bacteria are beneficial as they enhance the nutrient status of vermicompost as well as improve the soil aeration and fertility.

LITERATURE CITED

1. Karmakar S, Brahmachari K, Gangopadhyay A, Choudhury SR. 2012. Recycling of different available organic wastes through vermicomposting. *E-Journal of Chemistry* 9(2): 801-806.
2. Zularisam W, Zahirah Z, Ideris Z, Syukri MM, Anwar A, Munaim A, Sakinah M. 2010. Production of biofertilizer from vermicomposting process of municipal sewage sludge. *Journal of Applied Sciences* 10: 580-584.
3. Sinha J, Chanchal KB, Arup G, Amit S. 2010. Efficacy of Vermicompost against fertilizers on Cicer and Pisum and on population diversity of N2 fixing bacteria. *Journal of Environmental Biology* 31: 287-292.
4. Yan YW, Nor Azwady AA, Zulkifli HS, Muskhazli M, Suraini AA, Teng SK. 2012. Enhancement of plant nutrient contents in rice straw vermicompost through the addition n of rock phosphate. *Acta Biologica Malaysiana* 1: 41-45.
5. Emperor GN, Kumar K. 2015. Microbial population and activity on vermicompost of *Eudrilus eugeniae* and *Eisenia fetida* in different concentrations of tea waste with cow dung and kitchen waste mixture. *International Journal of Current Microbiology and Applied Sciences* 4(10): 496-507.
6. Lee KE. 1985. Earthworms: Their ecology and relationships with soils and land use. Academic Press Sydney Australia. pp 411.
7. Edwards CA, Bohlen PJ. 1996. *Biology and Ecology of Earthworms*. Chapman and Hall London.
8. Chaioui I, Zibiliske M, Ohno T. 2003. Effects of earthworm casts and compost on soil microbial activity and plant nutrient availability. *Soil Biology and Biochemistry* 35: 295-302.
9. Pramanik P, Ghosh GK, Ghosal PK, Banik P. 2007. Changes in organic-C N P and K enzyme activities in vermicompost of biodegradable organic wastes under liming and microbial inoculants. *Bioresource Technology* 98: 2485-2494.
10. Doube BM, Stephens PM, Davorena CW, Ryderb MH. 1994. Interactions between earthworms beneficial soil microorganisms and root pathogens. *Applied Soil Ecology* 1: 3-10.
11. Suthar S. 2010. Recycling of agro-industrial sludge through vermitechnology. *Ecological Engineering* 36: 1028-1036.
12. Terhivuo J, Saura A. 1993. Genic and morphological variation of the parthenogenetic earthworm Aporrectodea-rosea in southern finland Oligochaeta Lumbricidae. *Annales Zoologici Fennici* 30(3): 215-224.
13. Vijayan TM, Middha SK, Usha T. 2012. Morphological and histological studies on the Vermicomposting Indian earthworm *Eudrilus eugeniae. World Journal of Zoology* 7(2): 165-170.
14. Pattnik S, Reddy MV. 2010. Nutrient status of vermicompost of Urban green waste processed by three earthworm species-*Eisenia fetida Eudrilus eugeniae* and *Perionyx excavates. Applied and Environmental Soil Science*, Article ID 967526. pp 13.
15. Allen GN. 1953. *Experiments in Soil Bacteriology*. Burgess Publishing Co. Minneopolis Minn. USA. pp 127.
16. Selvi CP, Koilraj AJ. 2015. Bacterial diversity in compost and vermicompost of cotton waste at Courtallam Nellai District in Tamil Nadu India. *International Journal of Current Microbiology and Applied Sciences* 4(9): 582-585.
17. Devi HS, Vijayalakshmi K, Pavana Jyotsna K, Shaheen SK, Jyothi K, Surekha RM. 2009. Comparative assessment in enzyme activities and microbial populations during normal and vermicomposting. *Journal of Environmental Biology* 30(6): 1013-1017.
18. Domínguez JJ, Edwards CA. 2011. Biology and ecology of earthworms species used for vermicomposting. *In*: (Eds) Edwards CA, Arancon NQ, Sherman RL. Vermiculture technology: earthworms organic waste and environmental management. CRC Press, Boca Raton. pp 27-40.
19. Manyuchi M, Phiri A. 2013. Vermicomposting in solid waste management: A Review. *International Journal of Scientific Engineering and Technology* 2(12): 1234-1242.
20. Sequeira V, Chandrashekar JS. 2015. Vermicomposting of biodegradable municipal solid waste using indigenous *Eudrilus* sp. earthworms. *International Journal of Current Microbiology and Applied Sciences* 4: 356-365.
21. Sobana K, Agnes Sharmila M, Jegadeesan M. 2016. A review on vermicomposting of bio waste using different earthworm species. *Journal of Environmental Science Computer Science and Engineering and Technology Sec A* 5(1): 193-200.
22. Gupta C, Prakash D, Gupta S, Nazareno MA. 2019. Role of vermicomposting in agricultural waste management. (Eds) S. Shah. Sustainable Green Technologies for Environmental Management. Springer Nature Singapore Pte Ltd.

23. Curry JP, Schmidt O. 2007. The feeding ecology of earthworms: A review. *Pedobiologia* 50: 463-477.
24. Kaur A, Singh J, Vig AP, Dhaliwal SS, Rup PJ. 2010. Composting with and without *Eisenia fetida* for conversion of toxic paper mill sludge into soil conditioner. *Bioresource Technology* 101: 8192-8198.
25. Natarajan N, Gajendran M. 2014. Vermiconversion of Paper mill sludge for recycling the nutrients using earthworm *Eudrilus eugeniae. International Organization of Scientific Research Journal of Environmental Science Toxicology and Food Technology* 8 (9): 06-11.
26. Biruntha M, John Paula JA, Mariappan P. 2013. Vermicultural and molecular characterization of composting endemic earthworms. *American Journal of Research Communication* 1(3): 168-180.
27. Manyuchi M, Whingiri E. 2014. Effect of vermicomposting period substrate quantity cow dung composition and their interactions on *Eisenia Fetida* during vermicomposting. *International Journal of Current Microbiology Applied Sciences* 3(8): 1021-1028.
28. Kale RD, Bano K, Sreenivasa MN, Vinayak K, Bagyaraj DJ. 1998. In: Incidence of cellulolytic and lignolytic organisms in the earthworm worked soils. (Eds) GK Veeresh, D Rajagopal and C A Viraktamath. Pro Ml Zoo! Collg Bangalore. pp 659-665.
29. Parthasarathi K, Ranganathan LS. 1999. Longevity of microbial and enzyme activity and their influence on NPK content in Pressmud vermicasts. *European Journal of Soil Biology* 35: 107-113.
30. Tiwari SC, Tiwari BK, Mishra RR. 1989. Microbial populations enzyme activities and nitrogen - phosphorous - potassium enrichment in earthworm casts and in the surrounding soil of a pineapple plantation. *Biology and Fertility of Soils* 8: 178-182.
31. Peterson JC, Hendriksen NB. 1993. Effect of passage through the intestinal tract of detrivore earthworm *Lumbricus* sp. on the number of selected gram-negative and total bacteria. *Biology and Fertility of Soils* 16: 227-232.
32. Nechitaylo TY, Yakimov MM, Gudinho M, Timmis KN, Belogolova E, Byzov BA, Kurakov AV, Jones DI, Golyshin PN. 2010. Effect of the earthworms *Lumbricus terrestris* and *Aporrectodea caliginosa* on bacterial diversity in soil. *Microbial Ecology* 59(3): 574 587.