Vermicomposting of various solid agro-wastes using *Eisenia fetida* and identification of normal flora from its mid-gut

Aliyi Barko Juju

A Thesis submitted to the department of applied Biology, School of applied natural Sciences, presented in partial fulfillment of the requirement of a Degree of masters of Science in Biotechnology.

Office of Graduate Studies

Adama Science and Technology University

August 2022

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List of Abbreviations

SW: Solid Wastes

GHG: Green House Gases

CFU: Colony Forming Unit

PAHs: Polycyclic Aromatic Hydrocarbons

SPSS: Statistical Package for the Social Sciences

ANOVA: Analysis of Variance

NAHDIC: National Animal Health Diagnostics and Investigation Center

MALDI-TOF MS: Matrix Assisted Laser Desorption Ionization- Time of Flight Mass Spectroscopy

Abstract

Vermiculture composting is the process of producing the compost using earthworms to change the organic wastes in to high quality compost that consists mainly of worm cast in addition to decayed organic matter. This Research was aimed to perform vermicomposting of various solid agro-wastes using *Eisenia fetida* and identification of normal flora from its mid-gut. solid waste such as Kchat, paper, cow dung and vegetable wastes were collected and transported into composting room. *E. fetida* was collected from Awash Melkasa research center. For the screening of the normal flora, the samples were taken from the mid-gut of *E. fetida* at the beginning of the composting and at the end of the composting on 45^{th}day from each vermicomposting treatments. The Physico-chemical properties of wastes, isolation of the normal flora from the mid-gut of *E. fetida,* biochemical characterization of screened potential isolates, and optimization of potential bacterial isolates these obtained from the mid gut of *E. fetida* were conducted. The data were analyzed using Microsoft excel 2010 version, and origin 2019b version. During Physico-chemical analysis, minimum and maximum pH value was obtained. The highest (9.34±0.046) pH mean values for vermicomposting were obtained from vegetable composting due to *E. fetida.* A considerable amount of electrical conductivities were detected Kchat and vegetable vermicomposting processes. Total organic carbon (TOC) and total nitrogen (TN) contents were also detected for the vermicomposting processes. A 17 type of normal flora of *E. fetida* these suggested to assist biotransformation of these wastes were identified using Matrix assistant laser Matrix-assisted laser desorption/ionization-time of flight mass spectrometry (MALDI TOF MS). Certain of these obtained isolates were including .such as *Bacillus. Anthracis* BPW-1, *B. megaterium* BPW-4, *B. cereus* BKW-4, *B. flexus* BKW-31, *B. pumilus* BKW-3, *B. subtilis* BKW-5, *Enterobacter cloacae* EKW-5, *E. hormaechei* EKW-5, *Serratia rubidaea* SRVW-5 and *Staphylococcus hominis* SVW 51. Optimization for certain potential isolates which obtained from the mid gut of *E. fetida* were determined and glucose, maltose, mannitol, peptone, KNO_3 and Yeast extract (YE) were found to be employed for growth of *B. subtilis* BKW-5, *B. flexus* BKW-31 and *B. cereus* BKW-4.

Key words: Carbon source, *E. fetida,* isolates, vermicomposting and wastes

1. Introduction

1.1. Background of the study

Vermicomposting is the process of producing compost by utilizing earthworms to turn the organic waste into high-quality compost that consists mainly of worm cast in addition to decayed organic matter. Vermicomposting helps to convert the organic wastes (agro-wastes, animal manure and domestic refuse) into highly nutrient fertilizers for plant and soil (Abbasi, 2009). Vermicompost is a finely divided peat-like material with excellent structure, porosity, aeration, drainage and moisture-holding capacity (Ismail, 2005). Vermicompost, an organic fertilizer rich in N_2, P, K, micronutrients and beneficial soil microbes (nitrogen fixing and phosphate solubilizing bacteria and actinomycetes), is a sustainable alternative to chemical fertilizers, which is an excellent growth promoter and protector for crop plants (Martínez et al., 2012). Vermicompost is an important component of organic farming systems, because it is easy to prepare, has excellent properties and is harmless to plants. Vermicompost improves the physical, chemical and a biological property of the soil as well contributes to organic enrichment (Sinha, 2011).

Investigations on vermicomposting and its impact on vegetable production are necessary, especially as it can be used as a bio-fertilizer. Research on vermicomposting will provide farmers with an environment-friendly fertilizer and assist in promoting the agriculture sector towards a greener future. The use of such technology will help in cost management in agriculture which is increased in the recent years and has added to the burden of farmers in terms of chemical fertilizers and chemical pesticides. Consequently, the cost of production has increased many folds. Use of organic source of fertilizers like vermicompost could be an effective solution to the problem where it could substitute the chemical inputs in crop productivity and reduce the economic cost and on the other hand may also lead to organic produce which fetches higher price in the market. The increase in living standards around the world has created a growing demand for such organic produce, or cultivation using only natural pesticides and fertilizers, which are perceived to be healthier for consumers and environment friendly (Kaplan, 2016a).

Therefore, this research aims at characterization of the production of quality vermicompost from locally available organic waste materials using composting earthworm. Vermicomposting is a green technique used to produce organic compost from organic waste with the aid of specific earthworm species. The resulting compost is rich in nutrients that can improve plant health and fertility. This study will be conducted to produce organic compost using a developed vermicomposting (Kaplan, 2016b, 2016a), technique and to enhance and increase the exchangeable nutritional content in the soil for utilization in sustainable agriculture. Vermicompost is an essential element in organic agricultural systems, as it contains beneficial and useful properties for plants. It enhances the physical, chemical and biological properties of the soil and increases its organic content (Pace et al., 2000).

Earthworms are considered one of the primary tools for treating solid organic wastes, which consist of domestic, agricultural and animal-based wastes. *E. fetida* is one of the earthworm species that works efficiently in breaking down and decaying natural remains and turning these scraps into high-quality organic compost. It is capable of eating as much as half of its weight daily. The behavioral activity of earthworms (feeding, burrowing and casting) enhances the physical, chemical and biological properties of organic matter and soil, there by augmenting the growth of agricultural crops naturally and safely (Thakur et al., 2018). During the process of vermicomposting, vermiworms are used to transform organic wastes into a high-quality product from degraded organic matter and the dead bodies of vermiworms (Jawaher, 2020).

In addition, because of the humic acids in vermicompost, significant amounts of nutrients such as N, P, K, Ca and Mg accumulate in the shoots, roots and leaves of plants. Vermicompost is a brownish black substance with high porosity, aeration and water retention capacity. It is rich in micronutrients and soil beneficial microbes (nitrogen-fixing and phosphate-solubilizing bacteria and actinomycetes) and it is a sustainable alternative to chemical composts, as well as an excellent growth enhancer and plant crop protector (Sinha, 2011)..

Vermicomposting salvages human wastes & divert huge SW from Landfills with big advantage of great economic & environmental significance is that production of vermicompost from organic wastes divert huge amount of SW from ending up in the landfills also saving cost on waste disposal and reducing discharge of toxic leachate and gases and emission of greenhouse

gases (GHG) which occurs from waste landfills. Earthworms have the real potential to accelerate and enhance the natural biodegradation and decomposition of organic materials from 60 to 80% by promoting the growth of beneficial decomposer aerobic bacteria in the waste biomass. They host millions of decomposer microbes in their mid-gut and also act as an aerator, grinder, crusher, chemical degrader and a biological stimulator (Rajiv et al., 2010).

Another matter of considerable significance is that the earthworms stabilize the organic residues in the waste removing any harmful pathogens and toxic chemicals from the compost. They partially detoxify" and disinfect the end product which is nearly odorless. Several studies have found that earthworms effectively bio-accumulate or biodegrade several organic and inorganic chemicals including heavy metals, organ chlorine pesticide and polycyclic aromatic hydrocarbons (PAHs) residues in the medium in which it inhabits. In fact in the conventional composting technology which is thermophilic (temperature rising up to 55C) many beneficial microbes are killed and nutrient especially nitrogen is lost (due to gassing off of nitrogen) and the end product is more homogenous, richer in plant-available nutrients & humus and significantly low contaminants. Vermicomposting does not involve such use of energy; earthworms aerate the system constantly by burrowing actions, create aerobic conditions in the waste materials by their burrowing actions and inhibit the action of anaerobic micro-organisms which release foul- smelling hydrogen sulfide and mercaptans. High volumes of carbon dioxide (CO2), methane (CH4) and nitrous oxides (N_2O) is emitted from the conventional composting process especially in anaerobic conditions (Rajiv et al., 2010).

1.2. Statement of the Problem

For many cities in developing nations, dealing with the environmental costs of solid waste is a current phenomenal challenge (Wang & Nie, 2001). The enormous production of solid wastes coupled with poor management system result in a significant environmental degradation, thus the safe and environmentally harmonious management of solid wastes becomes a major issue for these nations. Currently, in all corners of the world managing different organic wastes at low capital and operational cost as well as in eco-friendly and energy saving basis has attracted much attention and among these methods, vermicomposting is one of the techniques that have been applied in various parts of the world (Sangwan et al., 2010). Vermicomposting which is alternatively called earthworm vermistabilisation, worm composting, or annelic consumption

(Wang *et al.,* 2006), is an earthworm based aerobic process which has a unique position in the domain of environmental engineering, as it is the only pollution control that uses a multicellular animal as the main bio-agent (Abbasi et al., 2009). In this process, energy rich and complex organic substances have been bio-oxidized and transformed into stabilized products by combined action of earthworms and microorganisms; hence earthworms play a considerable role by fragmenting and altering all biological activity of the waste (Degefe et al., 2016).

Being a developing country, Ethiopian cities and towns also suffer with the environmental costs of solid waste management and like other developing countries, the solid waste produced in Ethiopia are also dominated by food, paper and wastes from animal and vegetable origin which are wholly biodegradable. The organic nature of these wastes offers various biological management options such as vermicomposting instead of disposal to landfill sites, open dumping or any other environmentally risky waste management alternatives. Despite the fact that the vermicomposting is commonly used as a means of managing municipal solid wastes in various parts of the world; it has not been started in Ethiopia yet (Kendie, 2009).

However, the current economic growth, industrialization and urbanization throughout the country have led to the generation of large quantities of organic wastes which need marketable, environmentally sound and cost effective management system. It is now time for Ethiopian cities to think about biological waste treatment system like vermitechnology with the intervention of appropriate biological organisms. This study aims to explore and characterize the suitability and potential use of an epigeic earthworm species, *E. fetida* in managing commonly dumped solid wastes in the study area and to identify the pattern of chemical changes during the vermicomposting of these wastes. Due to its wide temperature, moisture and disturbance (handling) tolerant, *E. fetida* is among the best vermicomposting earthworm species (Degefe et al., 2016)..

Waste is a problem of the modern civilized society as they are facing the escalating socio-economic and environmental cost of dealing with current and future generation of mounting solid wastes (SW). Extensive use of chemical fertilizers and pesticides in agriculture negatively impacts the soil and plants as chemical and pesticide residues are present in nutritional products and they accumulate in the food web and environment. Therefore, developing innovative agricultural practices that employ organic composts and environmentally friendly products will

navigate the agricultural sector toward a greener future and production of wholesome and nontoxic food products at affordable prices year-round. In addition, innovative agriculture is required to meet the demands of governments and companies1. Therefore, this research aims at technology development and modifications for the production of quality vermicompost from locally available organic waste materials using composting earthworms (Loehr et al., 1984).

1.3. Significance of the Study

Vermicomposting is a green technique that produces vermicompost from different types of organic wastes using specific earthworm species. It helps farmers to reduce their use of chemical fertilizers and the overall production costs. Vermicompost is considered an alternative to chemical additives in agricultural crop production that reduces economic costs, while producing healthier organic products for consumers and enriching the environment. Therefore, organic agriculture is rapidly becoming a common theme among scientists and the agricultural society. Vermicompost plays a vital role in non-artificial systems such as organic agriculture, sustainable agriculture, or environmentally friendly agriculture, owing to its potential to improve the nutritional value of crops and enhance soil fertility.

A considerable portion of solid waste (SW) consist of Organic wastes that are biodegradable and can be vermicomposted into a highly nutritive bio-fertilizer, 4-5 fold more powerful than conventional composts and even superior to chemical fertilizers for better crop growth and safe food production. Another serious cause of concern today is the emission of greenhouse gases (GHG), methane (CH_4) & nitrous oxides (N_2O) resulting from the disposal of SW either in the landfills or from their management by conventional composting systems. Waste degradation & composting by earthworms is proving to be economically & environmentally preferred technology over the conventional microbial degradation & composting technology as it is rapid and nearly odorless process, reducing composting time by more than half and the end product is both disinfected and detoxified (Edwards & Bohlen, 1996).

1.4. Objectives of the study

1.4.1. General objective

- To study vermicomposting process against cost effective solid wastes using *E. fetida* and identification of normal flora from its mid-gut

1.4.2. Specific objectives

- To prepare vermicompost and analyze its physico-chemical characteristics
- To conduct biochemical test for normal flora these isolated from the mid-gut of *E. fetida*
- To characterize and identify potential bacterial normal flora from the mid-gut of *E. fetida*

1.5. Research questions

This research has tried to address the following research questions:

- What major procedures and parameters involved to characterize the vermicomposting and vermicompost?
- Which bacterial normal flora found in the mid-gut of .*E.fetida*?
- What major roles does bacterial normal flora have in vermicomposting?
- What are the morphological, biochemical and automated techniques of identifying them?

1.6. Scope of the study

This study emphasized on characterization of vermicomposting of solid wastes using *E. fetida,* isolation and biochemical characterization of potential bacterial normal flora from the mid-gut of *E. fetida*. This research didn‘t test the molecular characteristics of *E. fetida* worms and their bacterial normal flora and didn’t quantitatively assess the enzymatic activities of the isolated bacterial normal flora, but focused only on the qualitative assessment of their enzymatic activities.

2. Literature Review

2.1. Global movement for vermicomposting solid wastes (SW) to divert waste from Landfills

Large scale vermicomposting of SW including the sewage sludge on commercial scale is a movement going on to divert solid waste from ending up in the landfills (Sherman, 2018). Municipal councils and composting companies are also participating in vermicomposting business, composting all types of organic wastes on commercial scale and selling them to the farmers. This has dual benefits of cutting cost on landfill disposal of waste while earning revenues from sale of worms & vermicompost. First serious experiments for management of municipal/industrial organic wastes were established in Holland in 1970, and subsequently in England, and Canada. Later vermiculture were followed in USA, Italy, Philippines, Thailand, China, Korea, Japan, Brazil, France, Australia, Israel & Russia. U.S. has some largest vermicomposting companies and plants in world and States are encouraging people for backyard vermicomposting to divert wastes from landfills (Munroe, 2007a).

2.1.1. Some important global Studies on Vermicomposting Technology

It have been reported that (Visvanathan et al., 2005) vermicomposting in great details and found that most earthworms consume, at the best, half their body weight of organics in the waste in a day. *E. fetida* can consume organic matter at the rate equal to their body weight every day. Earthworm participation enhances natural biodegradation and decomposition of organic waste from 60 to 80% over the conventional aerobic and anaerobic composting. Given the optimum conditions of temperature (20-30^{o}C) and moisture (60-70%), about 5 kg of worms (~10,000) can vermin process about one of waste into vermicompost within 30 days which adopted from (Sinha, 2009). The vermicomposting also able to convert fly ash to increase the soil fertility that assisted for plant growth promotion (Bhattacharya et al., 2012). As it is also rich in nitrogen and microbial biomass it can be vermicomposted by earthworms. They found that 25% of fly-ash mixed with sisal green pulp, parthenium and green grass cuttings formed excellent feed for *E. fetida* and the vermicompost was higher in N_2, K and P contents than other commercial manures. The earthworms ingest the heavy metals from the fly-ash while converting them into vermicompost (Mohini et al., 1998; Rajiv et al., 2010).

2.1.2. Vermicomposting process in Ethiopia

The current economic growth, industrialization and urbanization throughout the country have led to the generation of large quantities of organic wastes which need marketable, environmentally sound and cost effective management system. Vermicomposting technology is new to Ethiopia. However, it has been reported that application of vermicompost in different locations in Tigray National Regional State doubled grain yield of several crops in comparison to the check (Edwards & Bohlen, 1996).

The appropriateness and efficiency of three earthworm species; *E. fetida*, *E. andrei* and *Dendrobaena veneta* was investigated in managing wastes of Rose and Carnation flowers and these worms were preferred for vermicompost because they are resilient earthworms that can be readily handled and tolerate wider moisture and temperature ranges (Dominguez and Edwards, 2011). It was hypothesized that these worms can effectively manage cut flower wastes and produce good quality of soil amendments. The suitability and potential use of an epigeic earthworm species like *E. andrei* and *E. foetida* against crop residues and different animal manures such as cow dung, goat manure, poultry manure and swine manure had been reported in Ethiopia and in other country (Rameshwar & Argaw, 2016).

2.2. Basic types of vermicomposting systems

Batch systems are ones in which the bedding and food are mixed, the worms added, and nothing more is done (except by the worms) until the process is complete. Continuous-flow systems are ones in which worms are placed bedding, where upon feed and new bedding are added incrementally on a regular basis. There are three basic types of vermicomposting systems of interest to farmers: windrows, beds or bins, and flow-through reactors. Each type has a number of variants. Windrows and bins can be batch or continuous-flow systems, while all flow-through systems, as the name suggests, are of the continuous-flow variety.

2.2.1. Windrows

2.2.1.1. Static pile windrows (batch)

Static pile windrows are simply piles of mixed bedding and feed (or bedding with feed layered on top) that are inoculated with worms and allowed to stand until the processing is complete. These piles are usually elongated in a windrow style but can also be squares, rectangles, or any other shape that makes sense for the person building them. They should not exceed one meter in height (before settling). Care must be taken to provide a good environment for the worms, so the selection of bedding type and amount is important.

2.2.1.2. Top-fed windrows (continuous flow)

Top-fed windrows are similar to the windrows described above, except that they are not mixed and placed as a batch, but are set up as a continuous-flow operation. This means that the bedding is placed first, then inoculated with worms, and then covered repeatedly with thin (less than 10 cm) layers of food. The worms tend to consume the food at the food/bedding interface, then drop their castings near the bottom of the windrow. A layered windrow is created over time, with the finished product on the bottom, partially consumed bedding in the middle, and the fresher food on top. Layers of new bedding should be added periodically to replace the bedding material gradually consumed by the worms.

The major disadvantages to this system are related to the winter conditions in which unlike the batch windrows described above, these windrows require continuous feeding and are difficult if not impossible to operate in the winter. In addition, if windrow covers are used, they must be removed and replaced every time the worms are fed, creating extra work for the operator. The advantages of top-feeding have mainly to do with the greater control the operator has over the worms' environment: since the food is added on a regular basis, the operator can easily assess conditions at the same time and modify such things as feeding rate, pH, moisture content, etc., as required. This tends to result in a higher-efficiency system with greater worm production and reproduction. Harvesting is usually accomplished by removing the top 10-20 cm first, usually with a front-end loader or tractor outfitted with a bucket (Bogdanov, 1996). This material will contain most of the worms and it can be used to seed the next windrow. The remaining material will be mostly vermicompost, with some unprocessed bedding. This can be used as is or

screened, with unfinished material put back into the process. This is essentially the system used by North America's largest vermicomposting facility, a 77acre operation run by American Resource Recovery in northern California that processes 300 tons of paper wastes per day.

2.2.1.3. Wedges (continuous flow)

The vermicomposting wedge is an interesting variation on the top-fed windrow. An initial stock of worms in bedding is placed inside a corral-type structure (3-sided) of no more than three feet or one meter in height. The sides of the corral can be concrete, wood, or even bales of hay or straw. Fresh material is added on a regular feeding schedule through the open side, usually by bucket loader. The worms follow the fresh food over time, leaving the processed material behind. When the material has reached the open end of the corral, the finished material is harvested by removing the back of the corral and scooping the material out with a loader. Using this system, the worms do not need to be separated from the vermicompost and the process can be continued indefinitely. During the coldest months, a layer of insulating hay or straw can be placed over the active part of the wedges. The corrals can be any width at all, the only constraint being access to the interior of the piles for monitoring and corrective actions, such as adjustment of moisture content or pH level. A corral width of about 6 feet, with space between adequate for foot travel, would be ideal. The ideal length will depend on the material being processed, the size of the worm population, and other factors affecting processing times (Haque, 2009; Munroe, 2007; Singh, 2014).

2.2.2. Stacked bins (batch or continuous flow)

One of the major disadvantages of the bed or bin system is the amount of surface area required. While this is also true of the windrow and wedge systems, they are outdoors, where space is not as expensive as it is under cover. Growing worms indoors or even within an unheated shelter is an expensive proposition if nothing is done to address this issue. Stacked bins address the issue of space by adding the vertical dimension to vermicomposting. The bins must be small enough to be lifted, either by hand or with a forklift, when they are full of wet material. They can be fed continuously, but this involves handling them on a regular basis (Beetz, 1998).

The more economical route to take is to use a batch process, where the material is pre-mixed and placed in the bin, worms are added, and the bin is stacked for a pre-determined length of time

and then emptied. This method is used by a number of professional vermicompost producers in North America. The main disadvantage of the stacked-bin system is the initial cost of set-up. It requires an unheated shelter, bins, a way to mix the bedding and feed, and equipment to stack the bins, such as a forklift. On a smaller scale, of course, this could all be done by hand. Another disadvantage arises when it comes time to harvest. As with the batch windrow systems, the worms are mixed in with the product and need to be separated. That requires either a harvester or another step in the process, where the material is piled so that the worms can migrate into new material (Haque, 2009; Munroe, 2007b).

3. Materials and Methods

3.1. Description of the Study Area

This Research was conducted in East Shoa Zone, in Adama city administration in ASTU Campus starting from October 2021 to September, 2022 G.C. The city was found in 1916 along Ethio-Djibouti rail way road. The city was about 100 km south east of Addis Ababa which occupies 136 km^2 of land in rift valley and stretches astronomically from 8^0 0 33 to 8^0 0 36 N and 39^0 11 57–39 21 15 E longitude. The city encountered the challenges of expansion of the squatter settlement and in appropriate waste disposal in alarming rate. The city has annual temperature of 21 -9^0C with rainfall of 847-14mm per annum. The city has sandy loam soil structure and has kola type of climate. Even though, it provides kola type of climate the surrounding Agricultural land provides high production of different cereals particularly in teff. Physically Adama is situated within the Wonji fault belt in the main structured system of the Ethiopian Rift Valley (Mulugeta, 2010; Tefera, 2010).

3.2. Sample collection and compost formulation

The Earth worm (*E. fetida*) was collected from Awash Melkas research centers. The Kchat straw, the waste paper and vegetable scraps were collected from the main Kchat market center of Adama city, Adama Boset Secondary School and the surrounding restaurants, respectively. The cow dung was procured from farmers around the city. These samples were transported into composting house. In this study, Kchat straw, waste paper, vegetable scraps and cow manure employed for vermicomposting processes. The formulation for vermicomposting processes was formulated according to Table-1. From all wastes, the non-biodegradable fractions such as plastic, rubber, polythene bags, wood, cardboard, glass and stones were separated and discarded manually by hand sorting. The waste materials and the cow manure used for the experiment were dried and chopped into small pieces before lying into experimental containers. All wastes were mixed with sliced cow manure in 3:1 ratio in dry weight basis. The earthworm species, *E. fetida* was obtained from Awash Melkasa Agricultural Research Center .and immediately placed in appropriate Laboratory environment (dark room, 25^{o}C with 55% humidity). The sample of (10g) was collected from the mid-gut of *E. fetida*, the serial dilution was conducted and transferred to the nutrient agar. For the screening of the normal flora, the samples were taken from the mid-gut of *E. fetida* at the beginning of the composting and at the end of the composting on 45th (fourty

fifth) day from each vermicomposting treatments. Temperature, humidity, pH, population of earthworms, the chemical and microbial characteristics of the vermicompost and isolates were recorded before and after composting, on 45thday.

Table 1. Treatments of the combination of solid wastes and number of earthworms for the vermicomposting process.

S,N	**Combination of the solid wastes based on weight proportion in (Kg) and number of earthworms (*E. fetida*)**
1	Kchat straw (3Kg) + Cow dung (1Kg) + 300 number of earthworms (*E. fetida)*
2	Vegetable scraps (3Kg) + Cow dung (1Kg) + 300 number of earthworms (*E. fetida)*
3	Waste paper (3Kg) + Cow dung (1Kg) + 300 number of earthworms (*E. fetida)*
4	Kchat straw (3Kg) + Cow dung (1Kg) will be used without earthworm as the experimental control
5	Vegetable scrap (3Kg) + Cow dung (1Kg) will be used without earthworm as the experimental control
6	Waste paper (3Kg) + Cow dung (1Kg) will be used without earthworm as the experimental control

All wastes were mixed with the sliced cow manure in the 3:1 ratio (Table 1; combination) in dry weight basis and three hundred (300) earthworms for each experimental treatment. The cow manure served as the supplement and it also supposed to increase the nutrient quality of the final product.

3.3. Physico-chemical analysis of compost sample

The chemical and physical characterization of compost samples' property parameters were conducted at Melkasa Agricultural Research Center (MARI). The PH was measured using the soil to water suspension ratio 1:5 (w/v), the total Nitrogen was determined according to Kjeldahl method (Bremner, 1960). The organic carbon was determined following the methods of Walkley & Black, (1934). The available phosphorus was also determined according to the methods of

Olsen, (1954). The Electrical conductivity was also determined following the methods of the Boyacuse hydrometer methods (Campbell & Gee, 1986; Rhoades et al., 1990). Total of six (6) samples; three compost and three control wastes were taken to the soil fertility laboratory in MARI. The physico-chemical characterization of each six (6) sample was conducted in triplicate manner.

3.4. Isolation of bacterial normal flora from the mid-gut of *E.fetida*

The earthworms were collected from composting wastes. These earth worms were immediately transported into laboratory. sample 10gm samples was collected from the mid-gut of earth worm (*E fetida*) after dissecting using sterilized forceps. The serial dilution was conducted and transferred into nutrient agar. For the screening of the normal flora, the samples were taken from the mid-gut of *E. fetida* at the beginning of the composting and at the end of composting (on 45^{th} date of composting) The newly isolated bacterial normal floras were further purified using nutrient agar (NA). The pure isolates were sub-cultured every 15 days until used for morphological characterization, biochemical characterization and enzymatic analysis.

3.5. Morphological characterization

The morphological characteristics of newly isolated bacteria were determined by Gram staining. The isolates were plated on NA for the selective isolation of Gram- negative bacteria. The isolates were further plated on milk agar with cetrimide (g/1) (skim milk powder, 133.3; peptic digest of animal tissue, 3.33; sodium chloride, 1.67; yeast extract, 1.0; cetrimide, 0.4; and agar. 20), a selective medium for pseudomonas species. The bacterial isolates then were further characterized by biochemical tests (Skariyachan *et al*., 2015). The Gram staining method was also carried out (Beveridge, 2001). Briefly, a suspension of bacteria was placed on a glass slide and was heat fixed. A few drops of crystal violet (1.24g in 100ml water, w/v) were added to the specimen and allowed to stand for 0.5 min and then a few drops of Gram's iodine (a mixture of 0.33g iodine and 0.67g potassium iodide in 100ml water,w/v) were added directly to the crystal violet on the slide to act as a mordant for 0.5 min. The crystal violet- gram's iodine mixture was poured from the slide; the slide was washed rapidly with tap water followed by a gentle flow of decolorizing fluid (95% v/v ethanol in water) for 20 sec. Then the slide was again washed rapidly in tap water, and carbol fuchsin (safranin) was added to it for 1 min and finally it was ready for microscopy after rinsing with tap water and drying with filter paper.

3.6. Biochemical Characterization

Different biochemical tests were carried out to differentiate between the bacterial isolates. The biochemical tests such as indole test and methyl red test were carried out. Depending on the test results the bacteria were categorized to the different genera.

Indole test: Indole test was used to examine the ability of certain bacteria to decompose the amino acid tryptophan to Indole. A sterilized tube containing 4ml of tryptophan broth was inoculated with 18-24 h. culture and was incubated at 37^{o}C for 24 h. Then, 0.5 ml of kovac's reagent was added and presence or absence of a ring was observed (Darkoh et al., 2015).

Methyl red test: This assay was used to determine if an organism metabolizing glucose utilizes mixed acid fermentation pathway and produces strong acid end products (lactic, acetic, or formic) that were detected by the indicator methyl red. 5ml MR-VP broth tube was inoculated with the organism and was incubated at 35^{o}C for 48-72h. 2.5 ml of the broth culture was transferred to a fresh tube and inoculated with five drops of methyl red indicator, then the presence or absence of color change was observed (Bullock & Aslanzadeh, 2013).

3.7. Enzymatic analysis for newly isolated bacterial normal flora of earth worm (*E. fetida*)

Different enzymatic tests including the cellulase, pectinase and protease were carried out to differentiate between the enzymes producing potential of the bacterial isolates. Depending on the test results the bacteria were categorized to the different genera categories.

3.7.1. Cellulase test

Cellulase producing isolates were screened on carboxymethyl cellulose (0.5%) agar plate. To visualize the clearing zones, the plates were flooded with an aqueous solution of Congo red (1mg/ml) for 15 minute. The cellulolytic organism produced a clearing zone surrounding the colony due to the digestion of carboxymethyl cellulose (Apun et al., 2000).

3.7.2. Pectinase test

Mineral salts medium (MSM) (K_2HPO_4, 1.8 g; NH_4Cl, 4.0 g; $MgSO_4$. $7H_2O$, 0.2g; NaCl, 0.1 g; $FeSO_4$. $7H_2O$ 0.01 g in 1 L of dH_2O water) was prepared. A 1% pectin was added to MSM

medium. A loop full of pure inoculum was streaked on to the agar plates and incubated at 37^0C. Finally, zones of utilization for pectin were recorded for respective isolates (Lordelo et al., 2000)

3.7.3. Protease tests

Protease test was conducted according to the methods of (Ribitsch *et al.*, 2012) with little modification. Nutrient agar medium was prepared and supplemented with 1% skim milk. A loop-full pure isolates were taken and streaked onto medium and incubated at 37^0C for 24 hrs. Finally, zones of utilization were recorded for respective proteolytic isolates.

3.8. MALDI-TOF MS analysis for newly isolated normal flora from the mid-gut of *E. fetida*

Bacterial colonies grown on culture medium were directly placed on the MALDI target plate as a thin layer and allowed to air dry. Two microliters of 70% formic Acid (Merck, Germany) were applied to the sample layer (Ha *et al.* 2019; Haigh *et al*., 2011), allowed to dry and overlaid with 2µ1 of matrix solution consisting of 10 mg/m1,-cyano-4-hydroxy-cinnamic acid (Fluka, Switzerland) saturated in 2.5% tri-fluoro acetic acid (sigma – Aldrich, USA) and 50% acetonitrile (Honeywell Burdick & Jackson, fisher Scientific). The matrix and analyte were allowed to co-crystalize and the target plate was inserted into the MALDI-TOF MS instrument. Mass spectra were automatically acquired with MALDI-TOF MS operating in a linear positive mode by accumulating 1,000 laser shots in the m/z ranges 2,000 – 20,000 and 500 – 3,000 using Thinker bell LT (ASTA Inc., korea). External calibration of mass spectra were performed using recombinant *E. coli* extracts (ASTA Inc.,) in a protein mass range of 3,000 – 20,000 Da and peptide calibration standard II (Bruker Daltonics, Germany) in a small molecule mass range of 500 – 3,000 Da.

3.9. Optimization of newly isolated dominant normal flora from the mid-gut of *E. fetida*

To understand the physiological growth requirements and the optimum growth conditions of the isolated bacteria, three isolates BKW-5, BKW-31 and BPW-4 were selected and different growth factors were analyzed. For these isolates, optimum condition for carbon source, temperature, pH range, and Nitrogen source were performed. The data were taken in tripilicate manner..

PH determination:- Flasks with broth containing the optimum concentration of substrate and carbon source were taken and the pH of the broth was adjusted to different pH value (6.5, 7 and 7.5) using 1N HCl and 1N NaOH. The pure cultures obtained from mid-gut of earthworm (*E. fetida*) were inoculated at 37ºC. At the end of incubation period the cell free culture filtrate was obtained for use as enzyme source.

Temperature:-Production medium at pH7 was inoculated with overnight grown selected bacterial strains (BKW-5, BKW-31 and BPW-4). The broth was incubated at various temperatures (25, 30 and 37 ºC) for 24h. At the end of incubation period, the cell free culture filtrate was obtained and enzyme analysis.

Carbon and Nitrogen sources:-The effect of various carbon source such as glucose, mannitol and maltose at the concentration of 1 to 5% was examined in the production medium. :- Various nitrogen sources like yeast extract, peptone and ammonium sulphate were evaluated for their efficiency to grow by providing 0.5% peptone in the production medium.

3.10. Data analysis

The data were computed into Microsoft excel and analyzed statistically to calculate and determine CFU (m/1), for the newly isolated and the dominant bacterial normal flora of earthworm (*E. fetida*) that isolated from their mid-gut. During data analysis, standard deviation, standard error, one way ANOVA was used to analyze the parameters for CFU mg/ml and for the vermicompost.

4. Results and Discussion

4.1. Physico-chemical characterization of vermicomposting and biomass of *E. fetida*

In this study, the pH is found to be the important parameter which greatly influences the vermicomposting process. The minimum and maximum pH value was detected for vermicomposting solid wastes (Appendix File: Table S_1). A 7.11-7.78 pH ranged was detected for the controls vermicomposting processes while between 8.057±0.015 to 9.34±0.04583 pH range was observed for the vermicomposting treatment samples. The pH mean value for vermicomposting processes due to *E. fetida* against vegetable composting was found to be the highest (9.34±0.046) in this study. Considerable amount of pH value (8.20±0.09) was also detected for Kchat vermicomposting (Appendix File: Table S_1). In contrary to the present \study, lower pH value (5.9-6.1) (Rakkini et al., 2017) was recorded for vermicomposting of organic wastes. The pH rising could be due to earth worm activities, biological oxidation, and other chemical reaction during the vermicomposting processes using Vermiculture. A similar results were obtained from vermicomposting of Cow dung and Herbal pharmaceutical industrial waste (HPIW) with alkalophilic pH range (8.08 ±0.22 to 9.02 ± 0.01) (D. Singh & Suthar, 2012). The optimum and acceptable pH for vermicomposting processes were found to be between 5.5 to 8,5 (Yadav & Garg, 2011) which a similar report to the recent our results.

Various electrical conductivities (ECs) were recorded for Kchat, paper and vegetable vermicomposting processes. The minimum (4.8±0.01) and maximum (11.317±0.075) ECs were detected for Kchat and vegetable vermicomposting that blended with cow dung in the presence of earth worm (Appendix File: Table S_1). However, the study conducted on vermicomposting of Donkey using epigeic earthworm *E. foetida* for electrical conductivities was found to be 3.91 ± 0.06 dS m−1 (Garg et al., 2006) which much lower than the current our results for vegetable vermicomposting using the same earth worm in the presence of cow dung combination. The rising of the ECs value for vegetable vermicomposting processes in our result may be attributed to mineralization these vegetable organic compounds.

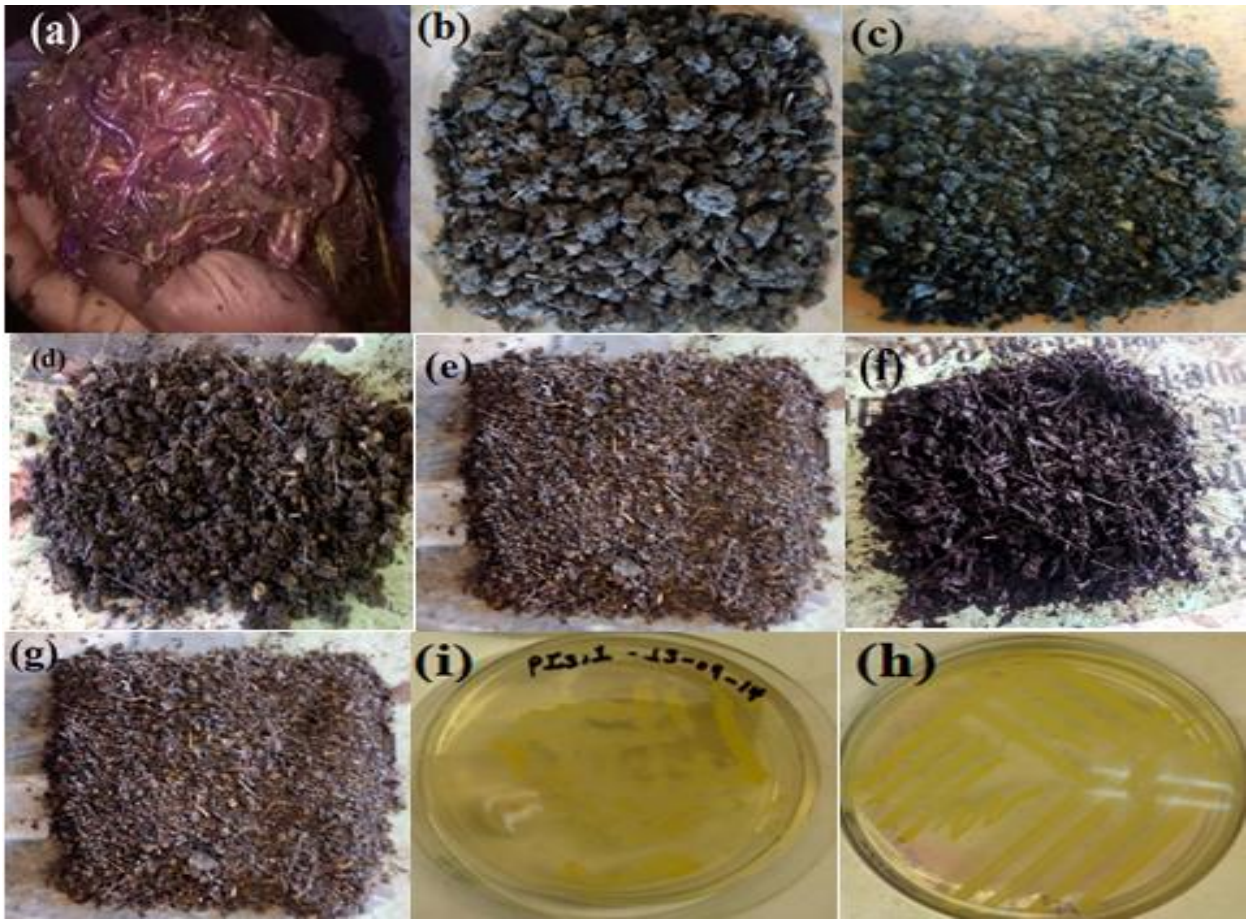


Figure 1: Earth worm, degraded Kchat, paper, vegetable wastes and purified bacterial isolates from these wastes (a) *E. fetida*, earthworm employed for vermicomposting processes for the present. study. (b) A vermicomposted of vegetable wastes on 22nd date. (c) A vermicomposted of vegetable wastes on 45th date. (d) A vermicomposted of paper wastes on 22nd date. (e) A vermicomposted of paper wastes on 45th date. (f) A vermicomposted of Kchat wastes on 22nd date. (g) A vermicomposted of Kchat wastes on 45th date. (i) Pure isolate obtained from the mid gut of *E. fetida* that provided with Kchat waste and cow dung. (h) Pure isolates of bacterial cells that obtained from the mid gut of E. fetida that feed on paper waste and cow dung.

The Total organic carbon (TOC) (%) and TN (%) were also found to be determinant factor for vermicomposting processes. The minimum (7.77±0.632 TOC%, 0.59±0.0TN%) and maximum (10.823±0.963 TOC%,, 1.0033±0.0058TN%) values were recorded for Kchat, paper and vegetable vermicomposting using vermiculture for TOC (%), and TN (%), respectively (Appendix File: Table S_1). Related amount of TK (%) were obtained from vermicomposting of various animal dung materials between 0.36 ± 0.04 to 0.73 ± 0.09 TK (%). However, 19.7 ± 0.65 to 40.7 ± 1.10 TOC (%) were recorded after vermicomposting processes using certain animal dung materials such as Buffalo, Camel, Cow dung, Donkey, Horse, Goats, and Sheep as the

main vermicomposting compositions (V. K. Garg et al., 2006) which is higher than the recent results for our TOC. In line with this study, it was reported that 33.85% of TOC was recorded for vermicomposting of floral waste (1325g) and cattle dung (500g) (Sharma et al., 2021).

The average weight of the Kchat, paper, and vegetable vermicomposting that ranged from 1.99 to 2.27 revealed that the wastes were approximately decomposed and degraded at conversion rate of 50% as compared to the weight of the beginning solid wastes as indicate in Figure 1b, c, d, e, f and g for these wastes. This was comparable with the previously report (Visvanathan et al., 2005) in which most earthworms are tend to consume, at the best, half their body weight of organics in the waste in a day. The same authors reported that given the optimum conditions of temperature (20-30$^{o}C^{0}$) and moisture (60-70%), about 5 kg of worms can be involved in vermicomposting processes within 30 days of composting. The present vermicomposting processes with considerable amount of TOC and TN% might be attributed for soil mineralization, improve soil fertility, and also increase soil aeration, soil porosity and hence this process can be used for plant growth promotion.

4.2. The average weight of compost, and average number of *E. fetida* population for each treatment after vermicomposting

E. fetida (Figure 1a) was able to significantly changed the physical and chemical properties of Kchat, paper and vegetable wastes as seen in Figure 1b, c, d, e, f and g on 22nd and 45th day of vermicomposting. In this study, it was found that the *E. fetida* able to turn organic waste into high quality compost. The lowest (338, 31.5%) and the highest number (387, 36%) of *E. fetida* were recorded for Vegetable and Kchat vermicomposting as indicated in the Table 2. From this result it has been realized that the Kchat vermicomposting is more suitable for *E. fetida* growth and hence it better for compost preparation. However, the population of *E. fetida* is lower for Vegetable vermicomposting indicating that the reproductive rates of this earth worm considerably less with more composting percentage (Table 2). From the Table 2, it was observed that the paper waste was the second suitable material for the preparation of vermicomposting with considerable amount of *E. fetida* population after the wastes treatment. It was indicating that vermicomposting technology is the suitable tools for bioconverting of these negligible solid wastes to agronomically important products that suitable for plant growth. In line with this study, it reported that *E. fetida* able to degrade Kchat wastes (Rameshwar & Argaw, 2016), paper

waste (Mathivanan et al., 2017) and vegetable solid waste (Suthar, 2009) and turn them into suitable composting. In this study, *E. fetida* population increased from 300 to 385 which less after 45 dates of vermicomposting processes on Kchat wastes which much lower *E. fetida* population when compared to vermicomposting processes on pig slurry with earth worm population is 5- and 8-fold increase over (Aira & Domínguez, 2008) the initial population of 500 mature earthworms after 36 weeks. These *E. fetida* population are able degrade Kchat, paper and Vegetable wastes and transforms theses wastes into useful vermicompost by mineralizing and modifying these wastes' biological, chemical and physical status which is a similar prediction to Lazcano et al., (2008).

As indicated in Table 3, the greatest weight (0.573g, 38.79% of the worms' weight) was recorded *E. fetida* that feed on Kchat wastes. This indicates that the Kchat wastes were easily degraded and decomposed by the *E. fetida* and suitable source for vermicompost preparation. Accordingly, the greater weight of worms (0.464gm, 31.42%) from the paper compost indicated that the paper wastes were the second degradable and suitable for the earth worms for conversion to compost and earthworm biomass among the treatment wastes. The smaller weight of the worms from the vegetable scrap wastes (0.197g, 13.34%) indicated that comparatively the vegetable wastes were not easily degraded and assimilated in to the earthworm's biomass.

'Table 2: The average weight and percentage of the compost produced, the average number and percentage of *E, fetida* population from each treatment after vermicomposting.

S.N.	Type of composting	Average weight and percentage of compost		Average number and percentage of *E. fetida* population	
1	Kchat vermicomposting	2.2 Kg	34.6%	387	36
2	Paper vermicomposting	1.99 Kg	30.6%	349	32.5
3	Vegetable vermicomposting	2.27 Kg	34.9%	338	31.5

Table 3. The average weight and percentage of the 20 dissected earthworms from each waste treatment

S.N.	Sample source	Average worms' weight in (gm)	Percentage
1	Vegetable Com	0.197	13.34
2	Kchat com	0.573	38.79
3	Paper Com	0.464	31.42
4	Before Composting	0.243	16.45

4.3. Biochemical test and evaluation of enzyme activities for these bacterial isolates obtained from mid gut of *E. fetida*

Certain bacterial isolates were obtained (Figure 2a-d) from the earth worms (*E. fetida*) was that collected from Kchat, paper and vegetable vermicomposting wastes. These bacterial isolates were found to be Gram positive (Figure 2a-d) and negative. There morphology after Gram staining was also found to be long rod-shaped.

4.4. Biochemical characterization of isolated bacterial normal flora

Physiological and the enzymatic tests were also used for the identification process. Out of the seventeen (17) isolates, 12 were found to be *Bacillus*, 3 isolates were *Enterobacter* as indicated in Table 4. In the study, one of *Staphylococcus* sp. and *Serratia* sp. were also predicted.

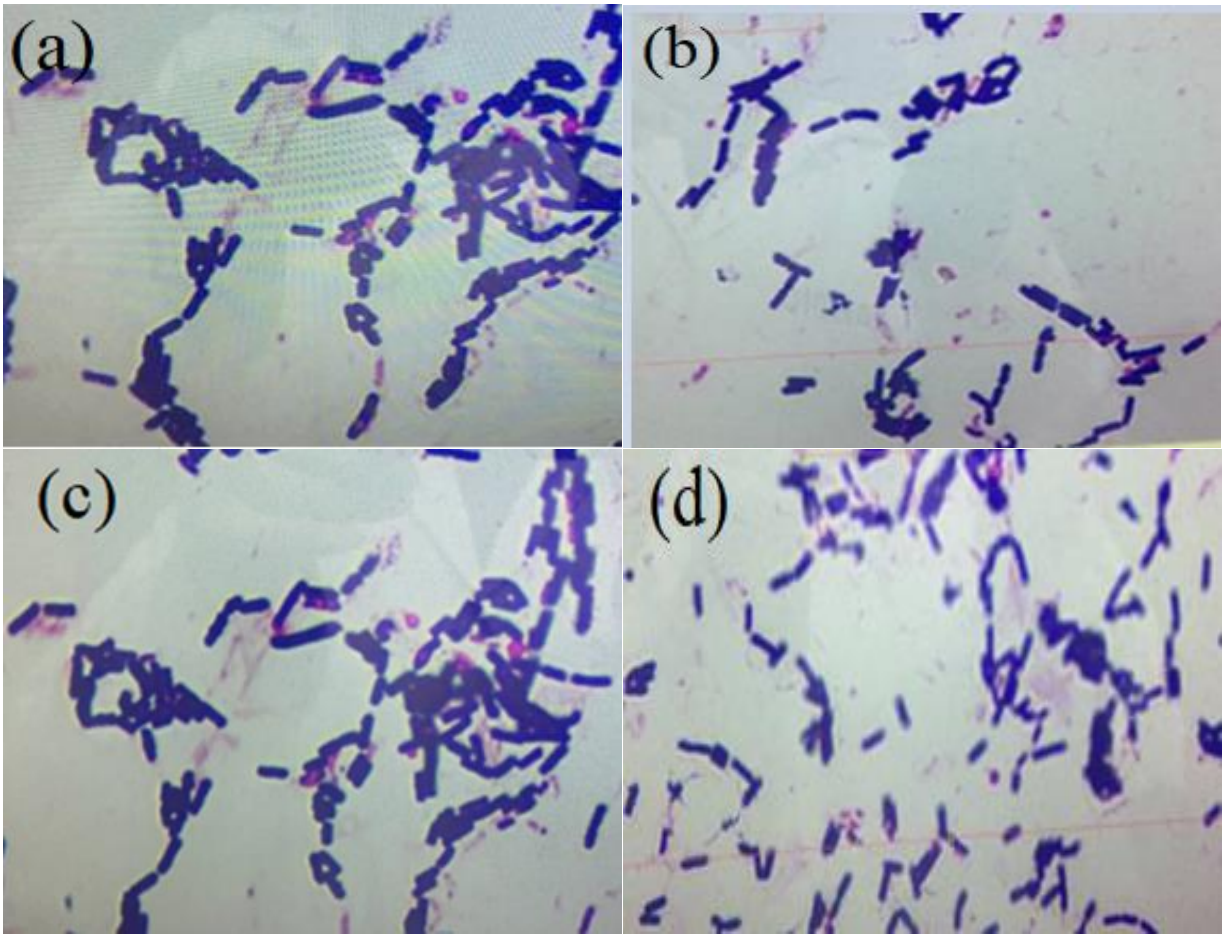


Figure 2. Gram stained pure isolates that were later subjected for by MALDI-TOF MS. (a) Isolate obtained from the mid gut of *E. fetida* feed on paper waste and found to be *B. flexus* BKW-31 when identified with MALDI-TOF MS. (b) Gram positive rod shaped isolate obtained from the mid gut of *E. fetida* that feed on Kchat waste and recognized to be *Bacillus flexus* BKW-31 after it was identified with MALDI-TOF MS. (c) Gram positive and rod shaped isolate which obtained from the mid gut of *E. fetida* that fed on Kchat wastes and found to be *Bacillus cereus* after it had been identified with MALDI-TOF MS. (d) A rod shaped Gram positive isolate that obtained from the mid gut of *E. fetida* that fed Kchat wastes and found to be *B. subtilis* BKW-5 after it had been identified with MALDI-TOF MS.

Table 4: Biochemical characteristics, enzymatic activities of bacteria isolated from mid gut of E. fetida that fed on Kchat, paper and vegetable wastes

S.N.	*List of isolates*	*Gram staining*	*Indole test*	*MR test*	**Enzyme activities**			**MALDI TOF MS**
					Cellulase test	Pectinase test	Protease test	
1	BKW-4	+ve	+ve	-ve	-ve	-ve	-ve	*B. cereus*
2	BKW- 3	+ve	+ve	-ve	-ve	-ve	-ve	*B. pumilus*
3	BKW-5	+ve	+ve	+ve	+ve	-ve	+ve	*B. subtilis*
4	BKW-31	+ve	+ve	-ve	+ve	+ve	-ve	*B. flexus*
5	BKW-41	*+ve*	*+ve*	*+ve*	*+ve*	*-ve*	*+ve*	*B. subtilis*
6	BKW-51	*+ve*	*+ve*	*+ve*	*+ve*	*-ve*	*+ve*	*B. subtilis*
7	EKW-5	-ve	+ve	-ve	-ve	+ve	-ve	*E. cloacae*
8	EKW-5	-ve	+ve	-ve	-ve	+ve	-ve	*E. hormaechei*
9	BPW-1	+ve	+ve	-ve	+ve	+ve	-ve	*B. anthracis*
10	BPW-4	+ve	+ve	-ve	+ve	+ve	-ve	*B. megaterium*
11	BPW-5	+ve	+ve	+ve	+ve	+ve	+ve	*B. subtilis*
12	BVW-5	+ve	+ve	+ve	+ve	-ve	+ve	*B. subtilis*
13	SRVW-5	-ve	-ve	-ve	+ve	+ve	+ve	*S. rubidaea*
14	BVW-51	*+ve*	*+ve*	*+ve*	*+ve*	*-ve*	*+ve*	*B. subtilis*
15	SRVW 51	*-ve*	*-ve*	*-ve*	*-ve*	*+ve*	*-ve*	*E. cloacae*
16	SVW 51	-ve	+ve	-ve	-ve	+ve	-ve	*S. hominis*
17	BVW-5	-ve	-ve	-ve	-ve	+ve	-ve	*E. hormaechei*

4.5. Identification of Isolated normal flora bacteria by MALDI-TOF MS

The MALDI-TOF MS analysis of the isolated bacteria carried out at NAHDIC and showed the various bacterial normal flora these occupying themed gut of *E. fetida* these feed on Kchat, paper and vegetable wastes (Table 5). As indicated in the Table 5, a 27 Bacterial isolates were identified. They are mostly *Bacillus* species. Other bacterial species such as *Enterobacter*, *Serratia*, and *Staphylococcus* were also identified using MALDI TOF MS from the mid gut of *E. fetida* these feed on various solid agro-wastes (Table 5).

The MALDI-TOF MS bio-typing result indicated that the *E. fetida* harbored various bacterial floras among which the *Bacillus* species were dominant micro-flora. The microbial cells might be employed to biodegrade these wastes that ingested by *E. fetida in their mid-gut* using various enzymes these secreted by these bacterial isolates. From this study, it can be realized that the joint action of these bacterial isolates and *E. fetida are* able to biological stabilize and transform organic compound found in these wastes into more suitable composts that employed for growth plant promotion. These isolates were also identified to the species levels as *B. anthracis* BPW-1,

B. megaterium BPW-4, *B. cereus* BKW-4, *B. pumilus* BKW-3, *B. subtilis* BKW-5, *E. cloacae* EKW-5, *E. hormaechei* EKW-5, and *S. rubidaea* SRVW-5 from the mid gut of *E. fetida, that fed* Kchat, paper, and vegetable scrap in the presence of cow dung as a supplements (Table 1) for vermicomposting processes. Similarly, bacterial species such as *B. marisflavi, B. pumilus, B. infantis* (Abu-Dieyeh et al., 2019) and *B. subtilis, B. cereus, B. atrophaeus* (Alsayegh et al., 2021) were identified from their environments and were identified by using MALDI-TOF MS to the strain levels. Certain unidentified isolates were also depicted in Appendix File: Table S_1 in which their score value is less that 2 indicating that these isolates may be noble strains.

Table 5: MALDI-TOF MS analysis for bacterial flora species obtained from Kchat wastes fed *E.fetida*

S.N	ID for newly isolates	List of bacterial isolates	Score Value	S.N	ID for newly isolates	List of bacterial isolates	Score Value
1.	BKW-4	*B. cereus*	2.2	10	BPW-5	*B. subtilis*	1.89
2.	BKW-3	*B. pumilus*	1.78	11	BMEP-5	*B. subtilis*	2.2
3.	BKW-5	*B. subtilis*	1.99	12	BVW-5	*B. subtilis*	2.06
4.	BKW-31	*B. Flexus*	2.26	13	SRVW-5	*E. cloacae*	1.91
5.	BKW-41	*B. subtilis*	2.14	14	SRVW-5	*S. rubidaea*	2.14
6.	BKW-51	*B. subtilis*	1.86	15	BVW-51	*B. subtilis*	2.05
7.	EKW-5	*E. hormaechei*	2.31	16	SRVW 51	*E. cloacae*	2.19
8	BPW-1	*B. anthracis*	2.3	17	SVW 51	*S. hominis*	2.22
9	BPW -4	*B. megaterium*	1.72				

Key note: BKW-1 indicates *Bacillus* isolate obtained from Kchat waste and SVW-51 indicates Staphylococcus isolate obtained from Vegetable waste. And EKW indicates *Enterobacter* isolate obtained from Kchat waste. BPW indicates *Bacillus* isolate obtained from paper waste. BMEP-*Bacillus* isolate obtained from the mid gut of fetida, BVW indicates *Bacillus* isolate obtained from vegetable waste. EIOVW indicates *Enterobacter* isolate obtained from vegetable waste, SRVW indicates *Serratia isolate* obtained from vegetable waste and SVW indicates *Staphylococcus* isolate obtained from vegetable waste

4.6. Enzymatic activities for *E. fetida* mid gut newly isolated bacterial normal flora

The *E. fetida* mid gut bacterial normal flora were shown positive result for cellulose, protease and pectinase with various clear zones (CZ) and clearing index (CI) (Table 6). As it has been observed in Table 6, the minimum (10mm) and maximum (13mm) clear zone was detected for *S. rubidaea* SRVW-5 and *B. flexus* BKW-31 with 3 and 3.6 clearing index against cellulose, respectively. The presence of this enzyme in these bacterial isolates may be employed for biotransformation of the cellulose which is the chemical constitute of paper waste (Bayer & Lamed, 1992) and Kchat (*Catha edulis*) (Gabriel et al., 2021) wastes within a mid-gut of *E. fetida* for preparation mature compost.

E. fetida with various CZ and CI. The minimum and the maximum CZ for pectinase activities were obtained from *S. rubidaea* (8mm) and B. *megaterium* BPW-4 (13 mm) or *B. flexus* BIOKW-31 (13mm) with 2.6 and 3.6 or 3.1 CI, respectively. The presence of pectinase enzyme in these isolates (Table 6) might be contributed for biodegradation of pectin that existed within paper pulp, Kchat and vegetable wastes (Müller-Maatsch et al., 2016). Certain enzyme such as acid phosphatase, alkaline phosphatase, amylase, cellulase, endoglucanase, nitrate reductase, and pectinase (Tiwari et al., 2016) were reported from the mid gut of earthworm and suggested that these enzymes are able to degrade various complex organic compounds these containing cellulose, pectin, and others. Pectinase enzyme had been purified from *Bacillus subtilis* 15A-B92 that obtained from soil samples which is contrary to present our study in which the pectinase was also obtained from *B. subtilis* BPW-5, and *B. anthracis* BPW-1 these live within a mid-gut of *E. fetida*. This indicated that these isolates can be distinguished among each other to the strain and general levels. However, isolation of cellulolytic Pectinolytic and proteolytic bacterial isolates from the mid-gut of *E. fetida* worm could be the first report of this study.

Table 6: Clearing index of cellulase producing isolates of *E. fetida* mid-gut

S.N.	*List of isolates*	**Cellulase**			**Pectinase**			**Protease**			**MALDI TOF MS**
		CS (mm)	CZ (mm)	CI	CS (mm)	CZ (mm)	CI	CS (mm)	CZ (mm)	CI	
1	BKW-4	5	11	3.2	5	12	3.4	6	13	4	*B. cereus*
2	BKW- 3	5	10	3	5	11	3.2	6	12	3.1	*B. pumilus*
3	BKW-5	5	11	3.2	5	13	3.6	5	15	4	*B. subtilis*
4	BKW-31	5	13	3.6	6	13	3.1	5	11	3.2	*B. flexus*
5	BKW-41	5	12	3.4	5	13	3.6	5	14	3.8	*B. subtilis*
6	BKW-51	5	13	3.6	5	13	3.6	6	13	3.8	*B. subtilis*
7	EKW-5	5	9	2.8	5	10	3	5	9	2.8	*E. cloacae*
8	EKW-5	5	9	2.8	5	10	3	5	9	2.8	*E. hormaechei*
9	BPW-1	5	10	3	5	10	3	5	11	3.2	*B. anthracis*
10	BPW-4	5	11	3.2	5	13	3.6	5	11	3.2	*B. megaterium*
11	BPW-5	5	12	3.4	5	12	3.4	5	14	3.8	*B. subtilis*
12	BVW-5	5	11	3.2	5	13	3.6	5	14	3.8	*B. subtilis*
13	SRVW-5	5	10	3	5	8	2.6	6	9	2.5	*S. rubidaea*
14	BVW-51	5	12	3.4	5	13	3.6	5	15	4	*B. subtilis*
15	SRVW-51	5	10	3	5	9	2.8	6	9	2.5	*E. cloacae*
16	SVW-51	5	11	3.2	6	11	2.8	5	14	3.8	*S. hominis*
17	BVW-5	5	9	2.8	6	10	2.6	5	10	3	*E. hormaechei*

As illustrated in the Table 6, the recent highest zone of utilization for lipid due to protease was detected for *B. subtilis* BKW-5 (15mm) and followed by *S. hominis (14mm)*, normal flora of mid gut of E. *fetida* worms with 4 and 3.8 CI. These bacterial cells may produce more pectinase and hence employed for protein biotransformation during vermicomposting processes. Similarly, diversity of *Bacillus* species such as *B. amyloliquefaciens, Brevibacillus brevis, B. cereus, B. megaterium, B. subtilis, B. licheniformis, B. pumilus,* and *B. thuringiensis, these* (Barus et al., 2017) employed for protease biosynthesis were isolated from Fresh Indonesian Tempeh (traditional fermented food) and identified by using 16S rRNA gene sequence which similar to our isolates these identified based on protein profile, MALDI TOF MS analysis.

4.7. Optimization of growth conditions for the isolated bacteria

Bacterial growth conditions were optimized for three selected bacterial isolates (BAPI4, BAC15 and BACI31). Temperature, pH, Carbon source and Nitrogen source preferences of the bacterial isolates were tested. All results are presented as Mean ± Standard Deviation (mean ± SD) of triplicates of OD_{600nm} and Biomass as cell dry weight (CDW g/l) measurements. One way analysis of variance

(ANOVA) was applied to ascertain significant differences between the different growth conditions. Differences were considered to be significant when $P < 0.05$.

4.7.1. Optimization of PH

The minimum and maximum CDW (g/L) were detected for *B. megaterium* BPW-4 (0.00233±0.00061g/L) and *B. flexus* BKW-31 (0.01673±0.00103) at pH7 and pH7.5, respectively as illustrated in the Table 7 (Appendix File: Table S_3). The next higher CDW (g/L) was observed for *B. flexus* BKW-31 (0.01407±0.00021g/L) at pH7. This indicated that the recent isolates may degrade effectively these Kchat, paper and vegetable wastes within a mid-gut of *E. fetida* and simplify the vermicomposting processes at this optimum pH value (7.5). A related optimum pH for vermicomposting processes (Yadav & Garg, 2011) were recorded after industrial wastes had been managed by earth worms.

Table 7: Effect of pH value on bacterial isolates these obtained from the mid gut of *E. fetida against* CDW (g/L)

S.N.	pH value	Isolates from *E. fetida* mid gut	Biomass (CDW, g/L)		OD_{600nm}	
			Mean	SD	Mean	SD
	6.5	*B. subtilis* BKW-5	0.0051[a]	0.00115	1.81733	0.01124
1		*B. flexus* BKW-31	0.0065[a]	0.0005	1.63042	0.05005
		B. megaterium BPW-4	0.0071[a]	0.00945	1.2842	0.00244
2	7	*B. subtilis* BKW-5	0.00297	0.00093	1.697	0.00721
		B. flexus BKW-31	0.01407[bc]	0.00021	1.63167	0.00569
		B. megaterium BPW-4	0.00233[c]	0.00061	1.27933	0.00503
3	7.5	*B. subtilis* BKW-5	0.00273[c]	0.00064	1.68067	0.00551
		B. flexus BKW-31	0.01673[b]	0.00103	1.68467	0.0115
		B. megaterium BPW-4	0.00253[c]	0.00301	1.20567	0.00058

Key note: the same letter shown in the column indicated that there is no variation among these isolates in terms of mean value of CDW (g/L) at 0.05 test.

As indicated in the Table, the lower CDW (g/L) were also documented for *B. megaterium* BPW-4 (0.00253±0.00301, at pH7.5), and *B. subtilis* BKW-5 (0.00273±0.00064, at pH7.5). Great significant variations were observed between *B. subtilis* BKW-5 (pH6.5) & *B. flexus* BKW-31 (pH7.5) (*P=0.001*), and *B. megaterium* BPW-4 (pH6.5) & *B. flexus* BKW-31 (pH7.5) (P=0.003) in terms of CDW (g/L) (Appendix File: Table S_8). No variation observed between *B. subtilis* BKW-5 (pH6.5) & *B. flexus* BKW-31 (pH6.5) (P=0.618), *B. subtilis* BKW-5 (pH6.5) & *B. megaterium* BPW-4 (pH6.5) (P=0.478), *B. subtilis* BKW-5 (pH6.5) & *B. megaterium* BPW-4

(pH7) (P=0.329) and others as indicated in Table 7 (Appendix File: Table S_3). Various OD_{600nm} were also obtained from *B. subtilis* BKW-5, *B. flexus* BKW-31 and *B. megaterium* BPW-4 isolates these obtained from the mid gut of *E. fetida* (Appendix File: Table S_8).

4.7.2. Optimization of growth Temperature

Temperature is the other important parameters that able to influence the growth of bacterial isolates and hence the vermicomposting processes. The highest CDW (g/L) was obtained from *B. subtilis* BKW-5 (0.07133±0.00306g/L) at 30^{o}C (Table 8, Appendix File: Table S_4). The next higher CDW (g/L) was detected for *B. flexus* BKW-31 (0.00667±0.00042g/L) the same temperature. From this result it was realized that the optimum temperature for vermicomposting processes could be at 30^{o}C when bacterial and *E. fetida* jointly performed biotransformation for the recent solid agro-wastes such as Kchat, paper and vegetable wastes. In the previous study, the optimum temperature range for the earthworms vermicomposting process had been reported between 12 and 28^{o}C (Yadav & Garg, 2011).

Table 8: Effect of Temperature on bacterial isolates these obtained from the mid gut of *E. fetida against* CDW (g/L)

S.N.	Temperature	Isolates from *E. fetida* mid gut	Biomass (CDW, g/L)		OD_{600nm}	
			Mean	SD	Mean	SD
1	25	*B. subtilis* BKW-5	0.0044^a	0.00135	1.47564	0.10907
		B. flexus BKW-31	0.0065^a	0.0005	1.63367	0.05599
		B. megaterium BPW-4	0.0041^a	0.0009	1.03426	0.05702
2	30	*B. subtilis* BKW-5	0.07133^b	0.00306	1.76923	0.2068
		B. flexus BKW-31	0.00667^c	0.00042	1.64433	0.05435
		B. megaterium BPW-4	0.00643^c	0.0004	1.214	0.02443
3	37	*B. subtilis* BKW-5	0.00153^d	0.00092	0.98	0.02598
		B. flexus BKW-31	0.00227^e	0.00046	0.968	0.06729
		B. megaterium BPW-4	0.00217^e	0.00029	0.87	0.014

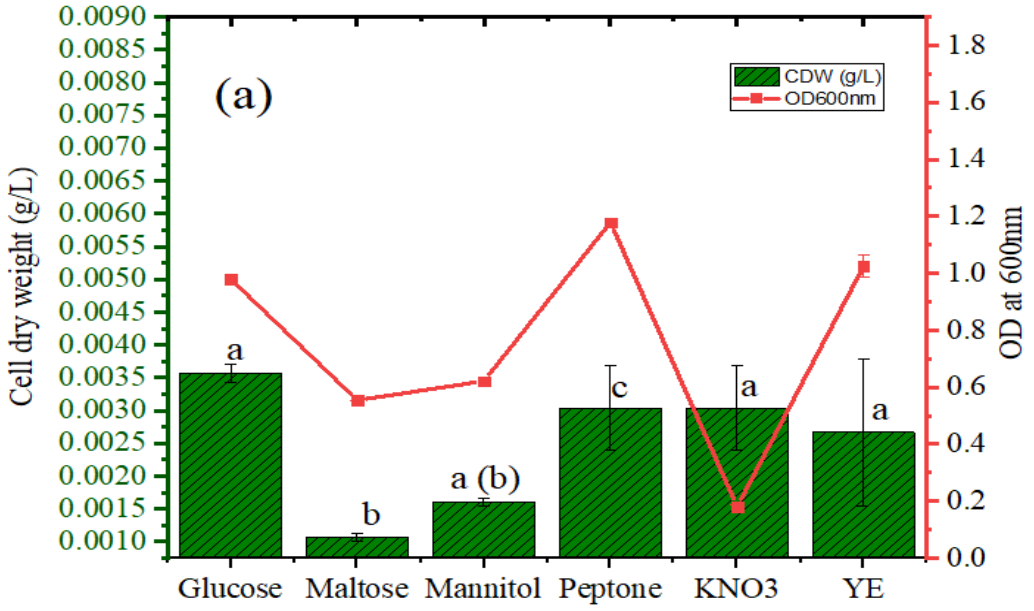
It was also reported that vermicomposting processes had been maintained between 20 to 22 °C (Saba et al., 2019) which is the lower temperatures when compared to the present our study. As indicated in the Table 8, significant variations in terms of CDW (g/L) were detected between *B. subtilis* BKW-5 (25^{o}C) & *B. megaterium* BPW-4 (37^{o}C) (P=0.04), *B. subtilis* BKW-5 (25 ^{o}C) & *B. flexus* BKW-31(37^{o}C) (P=0.48) and *B. subtilis* BKW-5 (30^{o}C) & *B. flexus* BKW-31 (37^{o}C) (P=0.000) (Appendix File: Table S_9). No variations were observed between *B. subtilis* BKW-5,

B. flexus BKW-31 and *B. megaterium* BPW-4 at various temperatures as illustrated in the Table 8.

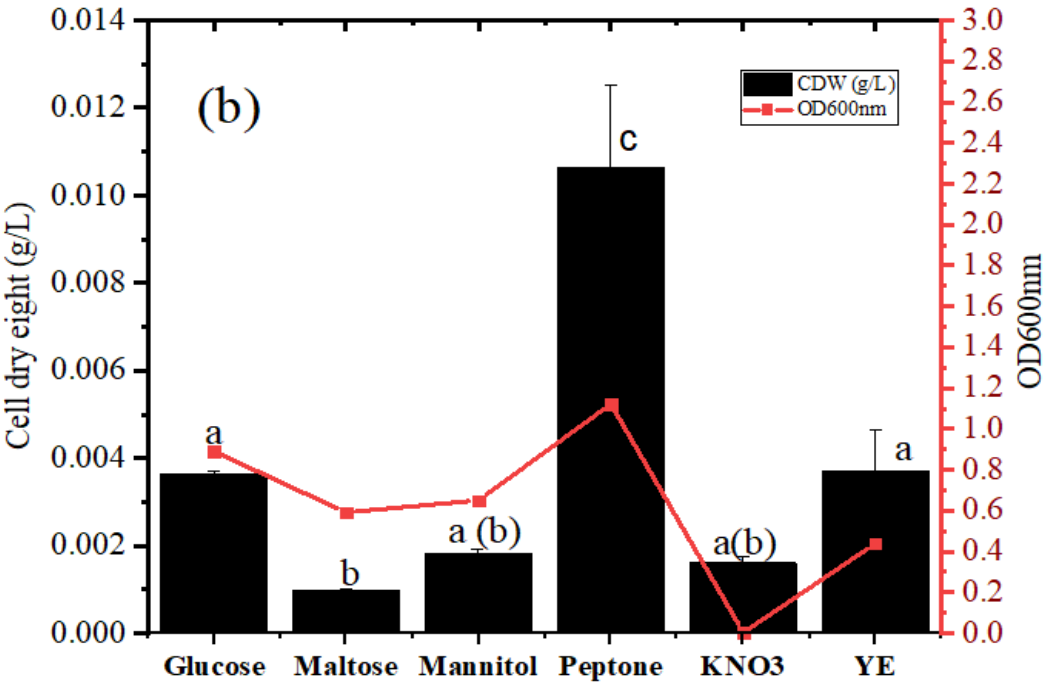
From the present study, it has been realized that other than various pH value, temperature is ale used to influence the vermicomposting processes. Factor such as type of substrate (organic residues), aeration, humidity, and the type of earthworm species (Pramanik et al., 2007) are used to determine the vermicomposting processes. This study has shown that the vermicomposting processes may be carried out in the mesophilic range since the recent optimum temperature for these mid gut of *E. fetida* isolates, which is a similar report to (Sharma et al., 2021). It was further confirmed that the biotransformation of organic compound was obtained at the mesophilic temperatures (Sharma et al., 2021)and this temperature is used to help earthworm and microbes under the aerobic condition for vermicomposting processes.

4.7.3. Carbon source and Nitrogen source against newly isolates

Certain carbon and nitrogen sources (Figure 3a-c) were found to be employed for growth of these isolates which obtained from mid gut of *E. fetid*. The contents of carbon and nitrogen contents of the recent employed carbon source for vermicomposting processes may be reduced which a similar results to (Garg et al., 2006) reports. The minimum and maximum CDW (g/L) were detected for *B. flexus* BKW-31 and *B. megaterium* BPW-4 against Maltose (0.00097±5.77E-05g/L) and KNO_3 (0.00507±0.00808g/L), respectively (Figure 3a-c, Appendix File: Table S_6). More CDW (g/L) was also recorded for Glucose (0.003667±0.0002517), Peptone (0.0056±0.00106g/L), and Yeast extract (YE) (0.0037±0.001601 g/L) against *B. subtilis* BKW-5 (Figure 3a, Appendix File: Table S_5), *B. megaterium* BPW-4 (Figure 3c, Appendix File: Table S_7), and *B. flexus* BKW-31 (Figure 3b, Appendix File: Table S_6), respectively. The present result indicated that these isolates are required nitrogen and carbon wastes for effective vermicomposting processes. Other than Kchat, paper, and vegetable wastes, these isolates could utilize other wastes these rich in carbon and nitrogen sources. It has been also realized that the nitrogen source are more suitable for these isolates than the carbon sources (Figure 3a-c). Less amount of CDW (g/L) has been obtained when these isolates were provided with maltose which is double sugar. It might be difficult for these isolates to break and maltose which is a double bond.



Effect of carbon and Nitrogen source on BKW-5 against CDW (g/L) and OD_{600nm}



Effect of carbon and nitrogen source on BKW-31 against CDW (g/L) and OD600nm

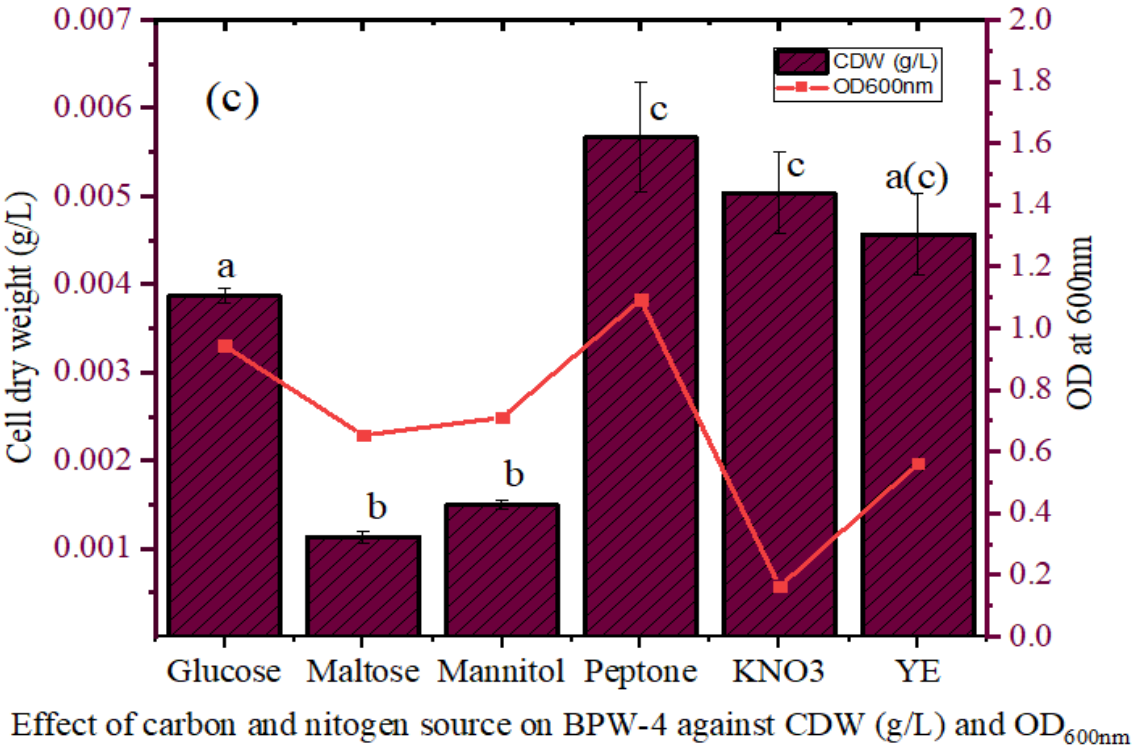


Figure 3: Effect of carbon and nitrogen source on newly isolated normal flora of earth worm. (a) *B. subtilis* BKW-5 isolates that obtained from the mid gut of *E. fetida* against various carbon and nitrogen source with considerable amount of CDW (g/L) and OD_{600nm}. (b) CDW (g/L) and OD_{600nm} for *B. flexus* BKW-31 isolate that obtained from the mid-gut of *E. fetida* against various carbon and nitrogen source. (c) *E. fetida* mid gut bacterial isolate (*B. megaterium* BPW-4) with CDW (g/L) and OD_{600nm} against various carbon and nitrogen source. Data represented mean ± SEM of three replicate (n = 3) of CDW (g/L) against various carbon and Nitrogen source. According to one way ANOVA, the CDW (g/L) has no significant variation at 95% confidence interval by using LSD test (P>0.05) for these isolates during growth on nutrient agar having the same latter on their respective bars.

The Kchat, paper, and vegetable wastes these mixed with cow dung are used in the form of carbon and nitrogen source for vermicomposting processes in the presence of *E. fetida* along with recently obtained its mid gut bacterial isolates such as *B. pumilus* BIKW- 3, *B. cereus* BIKW-4, *B. subtilis* BKW-5, *B. flexus* BKW-31, *B. megaterium* BPW-4, *E. cloacae* EIKW-5, *E. hormaechei* EIKW-5 , *S. hominis* SRIVW 51, and *E. hormaechei* SIVW 51, In line with this study, it was reported that paper mill wastewater sludge and cow dung (Negi & Suthar, 2018) had been employed for vermicomposting processes in the presence of earthworm (*E. fetida)* and brown-rot fungi

(*Oligoporus placenta*). The balanced supplying of these carbon and nitrogen reach solid agro wastes such as Kchat, paper and vegetable wastes these mixed with cow dung in the vermicomposting process used to attributed for microbial proliferation and hence contribute for degradation of complex organic polymers by producing certain enzymes (Table 4 & 6) along with *E. fetida* which is a similar report with (Mupambwa & Mnkeni, 2018). . Study conducted by (Huang et al., 2016) revealed that fresh fruit and vegetable wastes such as banana peels, cabbage, lettuce, potato, and watermelon peels were employed for optimum growth of *E. fetida* and lower carbon contents were obtained after vermicomposting process had been established. Similarly, in our study, it was recognized that after vermicomposting, the substrate completely turned to humus suggesting there is a lot of physical and chemical changes in the compost phenomena. There could be also a more biotransformation and mineralization during degradation of these wastes (i.e. Kchat, paper, and vegetable wastes) which rich carbon and nitrogen sources in the presence of *E. fetida* and its mid gut bacterial isolates. Variations were documented among *B. subtilis* BKW-5 (Appendix File: Table S_{10}), *B. flexus* BKW-31(Appendix File: Table S_{11}), and *B. megaterium* BPW-4 (Appendix File: Table S_{12}) in terms of carbon source such as and nitrogen source glucose, maltose, mannitol, peptone, KNO_3 and YE against their CDW (g/L).

5. Conclusion and Recommendation

5.1. Conclusion

The following conclusion is derived from the recent study. The physico-chemical results revealed that a pH ranged between 7.11-9.3 for both and experimental groups were obtained. The EC ranged between 5.44-11.32 for both controls treatments were detected. The TOC (%) between 9.21-10.83 was observed from the recent vermicomposting. The TN (0.80-1.00) was also obtained in this study for both control and experimental groups. In this study, between 11.58 to 17.52 C:N ratio were recorded from the vermicomposting. The number of earth worm (*E. fetida*) were found to be increased after vermicomposting for Kchat, Paper and Vegetable vermicomposting from 300 to 387, 300 to 349, and 300 to 338, respectively. The efficiency of vermicomposting process using *E. fetida* along with their normal flora of mid gut against Kchat, paper and vegetable wastes these mixed with cow dung found to be impressive. These prepared vermicompost may be employed to manage complex organic wastes and assist for plant growth after biotransformation due to *E. fetida* and their normal flora. The recently normal flora, these obtained from the mid gut of *E. fetida* were found to be *Bacillus. Anthracis* BPW-1, *B. megaterium* BPW-4, *B. cereus* BKW-4, *B. flexus* BKW-31, *B. pumilus* BKW-3, *B. subtilis* BKW-5, *Enterobacter cloacae* EKW-5, *E. hormaechei* EKW-5, *Serratia rubidaea* SRVW-5 and *Staphylococcus hominis* SVW 51 after identified with MALDI TOF MS. These isolates were suggested for biotransformation of Kchat, paper and vegetable wastes in the mid gut of *E. fetida*. These isolates were also found to be employed for secretion of various enzymes. Optimum growth condition for certain potential bacterial isolates such as *B. subtilis* BKW-5, *B. flexus* BKW-31, and *B. megaterium* BPW-4 were recorded. These bacterial isolated suggested to be employed for degradation of carbon and nitrogen wastes within the mid gut of *E. fetida.*

5.2. Recommendations

Based on the findings of this study, the following points were recommended for further scientific study.

- Because of different constraints, the full potential of the *E. fetida* bacterial flora in different solid wastes was not investigated leaving a gap in the knowledge of the

earthworm inhabiting bacterial normal flora. Therefore, further investigation of the multi-aspect potential of bacterial isolates has to be conducted.

- The enzymatic activities of the bacterial isolates should be quantitatively assessed to the level applicable in Industrial and agricultural production processes.
- The recently isolate can be recommended to use them for plant growth and for soil fertility..
- Certain potential isolate obtained from the mid gut of *E. fetida* were not identified by using MALDI TOF MS analysis. Further identification is recommended using more danced through but technology for these isolates since they might be noble isolates.
- Finally, the detailed genetic investigation and analysis of the *E. fetida* worms and their bacterial normal flora wre recommended to be re-examined. .

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7. Appendices

Table 1: The Physico-chemical parameters analysis result of the compost and control samples

S.N.	Sample code	Rep	pH	EC (dS/m)	%TOC	%TN	Av. P (ppm)
1	Vegetable Comp(V.Com)	1	9.39	11.24	9.13	0.59	25.18
		2	9.33	11.39	11.62	0.59	25.83
		3	9.30	11.32	10.21	0.59	27.99
Mean			9.34	11.32	10.32	0.59	26.34
SD			0.05	0.08	1.25	0.00	1.47
2	Kchat Compost (K.Com)	1	8.29	4.79	9.91	1.00	29.51
		2	8.11	4.81	11.83	1.01	27.89
		3	8.21	4.80	10.73	1.00	27.99
Mean			8.20	4.80	10.83	1.00	28.46
SD			0.09	0.01	0.97	0.00	0.91
3	Paper control (P.C)	1	7.78	5.44	9.21	0.80	24.64
4	Kchat control (K..Com)	1	7.23	7.37	10.47	0.87	22.16
5	Vegetable Control (V.C)	1	7.11	5.79	10.21	0.81	25.40
6	Paper compost (P.Com)	1	8.04	6.31	7.17	0.71	23.99
		2	8.07	6.37	8.43	0.72	24.64
		3	8.06	6.34	7.70	0.71	26.37
Mean			8.06	6.34	7.77	0.71	25.00
SD			0.02	0.03	0.63	0.00	1.23

Table 2: MALDI-TOF MS results of isolated normal flora from the mid-gut of *E.fetida*

Bruker MALDI Biotyper Identification Results

Run Info:

Run Identifier:	220827-1626-1011027865
Comment:	Ali second test
Operator:	Admin@MBT-WIN10
Run Creation Date/Time:	2022-08-27T17:30:15.443
Number of Tests:	15
Type:	Standard
BTS-QC:	passed
BTS-QC Position:	G7:0
Instrument ID:	8604832.05381
Server Version:	4.1.100 (PYTH) 174 2019-06-158_01-16-09

Result Overview

Sample Name	Sample ID	Organism (best match)	Score Value	Organism (second-best match)	Score Value
E5 (+) (B)	01 (Standard)	Enterobacter cloacae	1.91	Enterobacter cloacae	1.81
E6 (-) (C)	02 (Standard)	no peaks found	0.00	no peaks found	0.00
E7 (+++) (A)	03-S (Standard)	Bacillus flexus	2.26	Bacillus flexus	2.07
E8 (-) (C)	03-L (Standard)	No Organism Identification Possible	1.68	No Organism Identification Possible	1.63
E9 (+) (B)	04 (Standard)	Bacillus subtilis	1.99	Bacillus subtilis	1.96
E10 (+) (B)	05 (Standard)	Bacillus subtilis	1.86	Bacillus subtilis	1.71
E11 (+++) (A)	06 (Standard)	Bacillus subtilis	2.30	Bacillus subtilis	2.03

Result overview table--continued on next page

Result overview table--continued from previous page

Sample Name	Sample ID	Organism (best match)	Score Value	Organism (second-best match)	Score Value
E12 (+) (B)	07-B (Standard)	Bacillus cereus	1.73	**No Organism Identification Possible**	1.60
G1 (+) (B)	08 (Standard)	Bacillus subtilis	1.89	Bacillus subtilis	1.79
G2 (+++) (B)	09 (Standard)	Enterobacter hormaechei	2.31	Enterobacter cloacae	2.05
G3 (+++) (A)	10 (Standard)	Bacillus subtilis	2.14	Bacillus subtilis	1.95
G4 (+++) (A)	12-B (Standard)	Bacillus subtilis	2.08	Bacillus subtilis	2.05
G5 (+++) (A)	13-L (Standard)	Bacillus subtilis	2.05	Bacillus subtilis	2.04
G6 (+) (B)	13-S (Standard)	Enterobacter cloacae	1.93	Enterobacter cloacae	1.85
G7 (+++) (A)	BTS (BTS)	Escherichia coli	2.19	Escherichia coli	2.10

Result overview table--continued from previous page

Sample Name	Sample ID	Organism (best match)	Score Value	Organism (second-best match)	Score Value
E8 (+++) (A)	20644 (Standard)	Staphylococcus sciuri	2.06	Staphylococcus sciuri	1.95
E9 (+++) (A)	20645 (Standard)	Klebsiella oxytoca	2.17	Klebsiella oxytoca	2.17
E10 (+++) (C)	20646 (Standard)	Klebsiella oxytoca	2.22	Raoultella ornithinolytica	2.13
E11 (+++) (A)	20647 (Standard)	Exiguobacterium aurantiacum	2.13	Exiguobacterium sp[3]	1.74
E12 (-) (C)	20648 (Standard)	**no peaks found**	0.00	**no peaks found**	0.00
F1 (+++) (A)	20874 (Standard)	Bacillus anthracis	2.30	Bacillus anthracis	2.30
F2 (-) (C)	20875 (Standard)	**no peaks found**	0.00	**no peaks found**	0.00
F3 (+) (B)	20876 (Standard)	Bacillus pumilus	1.78	Bacillus pumilus	1.77
F4 (+) (B)	20877 (Standard)	Bacillus megaterium	1.72	**No Organism Identification Possible**	0.00
F5 (-) (C)	20878 (Standard)	**no peaks found**	0.00	**no peaks found**	0.00
F6 (-) (C)	20881 (Standard)	**No Organism Identification Possible**	0.00	**No Organism Identification Possible**	0.00
F7 (+++) (A)	20880 (Standard)	Staphylococcus hominis	2.22	Staphylococcus hominis	2.14
F8 (+++) (A)	20879 (Standard)	Serratia rubidaea	2.14	Serratia rubidaea	2.09
F9 (+) (B)	BTS (BTS)	Escherichia coli	1.99	Escherichia coli	1.89

Table 3 : Effect of pH value on certain normal flora of E. fetida and their respective CDW (g/L) and OD_{600nm}

	Biomass (CDW, g/L)							OD at 600nm					
PH	Isolates codes	R1	R2	R3	AV	SD	SEM	R1	R2	R3	AV	SD	SEM
6.5	BKW-5	0.004	0.005	0.0063	0.0051[a]	0.00115	0.00067	1.805	1.82	1.827	1.81733	0.01124	0.00649
	BKW-31	0.007	0.006	0.0065	0.0065 [a]	0.0005	0.00029	1.688	1.606	1.59727	1.63042	0.05005	0.0289
	BPW-4	0.018	0.0013	0.002	0.0071[a]	0.00945	0.00545	1.285	1.28614	1.28146	1.2842	0.00244	0.00141
7	BKW-5	0.004	0.0027	0.0022	0.00297	0.00093	0.00054	1.705	1.695	1.691	1.697	0.00721	0.00416
	BKW-31	0.014	0.0143	0.0139	0.01407[bc]	0.00021	0.00012	1.627	1.63	1.638	**1.63167**	0.00569	0.00328
	BPW-4	0.003	0.0022	0.0018	0.00233[c]	0.00061	0.00035	1.28	1.274	1.284	1.27933	0.00503	0.00291
7.5	BKW-5	0.003	0.002	0.0032	0.00273[c]	0.00064	0.00037	1.681	1.675	1.686	1.68067	0.00551	0.00318
	BKW-31	0.017	0.0176	0.0156	0.01673[b]	0.00103	0.00059	1.685	1.696	1.673	1.68467	0.0115	0.00664
	BPW-4	6E-04	0.001	0.006	0.00253[c]	0.00301	0.00174	1.20601	1.206	1.205	1.20567	0.00058	0.00034

Table 4: Effect of temperature value on certain normal flora of E. fetida and their respective CDW (g/L) and OD_{600nm}

	Biomass (CDW, g/L)							OD					
Temp	Isolates code	R1	R2	R3	AV	SD	SEM	R1	R2	R3	AV	SD	SEM
25	BKW-5	0.003	0.0057	0.0045	0.0044	0.00135	0.00078	1.60068	1.400048	1.4262	1.47564	0.10907	0.06297
	BKW-31	0.007	0.006	0.0065	0.0065	0.0005	0.00029	1.698	1.607	1.596	1.63367	0.05599	0.03232
	BPW-4	0.005	0.0032	0.0041	0.0041	0.0009	0.00052	1.00069	1.100095	1.00199	1.03426	0.05702	0.03292
30	BKW-5	0.072	0.074	0.068	0.07133	0.00306	0.00176	1.707	2	1.60069	1.76923	0.2068	0.1194
	BKW-31	0.007	0.0062	0.0068	0.00667	0.00042	0.00024	1.707	1.61	1.616	1.64433	0.05435	0.03138
	BPW-4	0.006	0.0065	0.0068	0.00643	0.0004	0.00023	1.231	1.225	1.186	1.214	0.02443	0.01411
37	BKW-5	0.001	0.001	0.0026	**0.00153**	0.00092	0.00053	0.995	0.995	0.95	0.98	0.02598	0.015
	BKW-31	0.002	0.0028	0.002	0.00227	0.00046	0.00027	0.992	1.02	0.892	0.968	0.06729	0.03885
	BPW-4	0.002	0.0025	0.002	0.00217	0.00029	0.00017	0.854	0.88	0.876	0.87	0.014	0.00808

Table 5: Effect of carbon and nitrogen sources on isolate *B. subtilis* BKW-5

BKW-5												
	Biomass (CDW, g/L)						**OD**					
CS for BKW-5	R1	R2	R3	AV	SD	SEM	R1	R2	R3	AV	SD	SEM
Glucose	0.0033	0.0038	0.0036	0.003667	0.0002517	0.0001453	0.978	0.981	0.98	0.98033	0.00153	0.00286
Maltose	0.0012	0.001	0.001	0.0011	0.0001732	0.0001	0.557	0.553	0.553	0.554	0.00173	0.00275
Mannitol	0.0016	0.0017	0.0015	0.001666	1.53E-04	8.82E-05	0.622	0.623	0.62	0.62233	0.00208	0.00696
	Biomass (CDW, g/L)						OD					
NS For BACI5	R1	R2	R3	AV	SD	SEM	R1	R2	R3	AV	SD	SEM
Peptone	0.0043	0.0026	0.0022	0.0031	0.0011533	0.000665	1.177	1.171	1.188	1.17955	0.00861	0.00498
KNO3	0.0048	0.001	0.0022	0.002766	0.0019424	0.0011214	0.191	0.169	0.183	0.181333	0.01158	0.00668
YE	0.0015	0.0074	0.0034	0.0041	0.0030115	0.0017386	0.95	1.064	1.063	1.02666	0.06552	0.03783

Table 6: Effect of carbon and nitrogen sources on isolate *B. flexus* BKW-31

CS and NS	**Biomass (CDW, g/L)**						**OD**					
CS	R1	R2	R3	AV	SD	SEM	R1	R2	R3	AV	SD	SEM
Glucose	0.0037	0.0035	0.0037	0.00373	0.000115	6.67E-05	0.891	0.89	0.892	0.892	0.0006	0.0003
Maltose	0.001	0.0009	0.001	0.00097	5.77E-05	3.33E-05	0.592	0.591	0.592	0.593	0.0006	0.0003
Mannitol	0.0018	0.002	0.0017	0.0018	1.00E-04	5.77E-05	0.65	0.652	0.65	0.652	0.0012	0.0007
	Biomass (CDW, g/L)						OD					
NS	R1	R2	R3	AV	SD	SEM	R1	R2	R3	AV	SD	SEM
Peptone	0.0084	0.0091	0.0144	0.01073	0.003281	1.19E-03	1.11	1.118	1.138	1.125	0.0113	0.0065
KNO_3	0,0021	0.0018	0.0014	0.00186660	0.0035117	2.03E-04	0.004	0.002	0.003	0.004	0.0006	0.0003
YE	0.0055	0.0032	0.0024	0.0037	0.001601	9.29E-04	0.422	0.444	0.455	0.445	0.0215	0.0124

Table 7: Effect of Carbon and Nitrogen sources on isolate *B. megaterium* BPW-4

	Biomass (CDW, g/L)						OD at 600nm					
CS for BPW-4	R1	R2	R3	AV	SD	SEM	R1	R2	R3	AV	SD	SEM
Glucose	0.0037	0.0039	0.004	0.0038	0.0001	5.77E-05	0.943	0.944	0.945	0.94467	0.00153	0.00088
Maltose	0.0012	0.001	0.0012	0.0011	0.00173	0.0001	0.654	0.652	0.651	0.65367	0.00116	0.00067
Mannitol	0.0016	0.0014	0.0015	0.0015	1.00E-04	5.77E-05	0.712	0.71	0.711	0.71133	0.00114	0.00065
	Biomass (CDW,g/L)						OD at 600nm					
NS for BAPI4	R1	R2	R3	AV	SD	SEM	R1	R2	R3	AV	SD	SEM
Peptone	0.0052	0.0049	0.0069	0.0056	0.00106	0.00061	1.053	1.116	1.11	1.09333	0.0342	0.01972
KNO3	0.0051	0.0042	0.0058	0.00507	0.00808	0.00047	0.15	0.16	0.177	0.16371	0.01434	0.00826
YE	0.0047	0.0053	0.0037	0.0045	0.00082	0.00047	0.566	0.563	0.558	0.56333	0.00404	0.00233

Table 8: Significant variation between variations pH value against CDW of certain isolate obtained from the mid-gut of E. foetida

Multiple Comparisons							
LSD							
Dependent Variable	(I) coding	(J) coding	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
CDW	CDW at pH6.5 for BKW-5	CDW at pH6.5 for BKW-31	-.00140000	.00275694	.618	-.0071921	.0043921
		CDW at pH6.5 for BPW-4	-.00200000	.00275694	.478	-.0077921	.0037921
		CDW at pH7 for BKW-5	.00213333	.00275694	.449	-.0036588	.0079255
		CDW at pH7 for BKW-31	-.00896667*	.00275694	.004	-.0147588	-.0031745
		CDW at pH7 for BPW-4	.00276667	.00275694	.329	-.0030255	.0085588
		CDW at pH7.5 for BKW-5	.00236667	.00275694	.402	-.0034255	.0081588
		CDW at pH7.5 for BKW-31	-.01163333*	.00275694	.001	-.0174255	-.0058412
		CDW at pH7.5 for BPW-4	.00256667	.00275694	.364	-.0032255	.0083588
	CDW at pH6.5 for BKW-31	CDW at pH6.5 for BKW-5	.00140000	.00275694	.618	-.0043921	.0071921
		CDW at pH6.5 for BPW-4	-.00060000	.00275694	.830	-.0063921	.0051921
		CDW at pH7 for BKW-5	.00353333	.00275694	.216	-.0022588	.0093255
		CDW at pH7 for BKW-31	-.00756667*	.00275694	.013	-.0133588	-.0017745
		CDW at pH7 for BPW-4	.00416667	.00275694	.148	-.0016255	.0099588
		CDW at pH7.5 for BKW-5	.00376667	.00275694	.189	-.0020255	.0095588
		CDW at pH7.5 for BKW-31	-.01023333*	.00275694	.002	-.0160255	-.0044412
		CDW at pH7.5 for BPW-4	.00396667	.00275694	.167	-.0018255	.0097588
	CDW at pH6.5 for BPW-4	CDW at pH6.5 for BKW-5	.00200000	.00275694	.478	-.0037921	.0077921
		CDW at pH6.5 for BKW-31	.00060000	.00275694	.830	-.0051921	.0063921
		CDW at pH7 for BKW-5	.00413333	.00275694	.151	-.0016588	.0099255
		CDW at pH7 for BKW-31	-.00696667*	.00275694	.021	-.0127588	-.0011745

		CDW at pH7 for BPW-4	.00476667	.00275694	.101	-.0010255	.0105588
		CDW at pH7.5 for BKW-5	.00436667	.00275694	.131	-.0014255	.0101588
		CDW at pH7.5 for BKW-31	-.00963333*	.00275694	.003	-.0154255	-.0038412
		CDW at pH7.5 for BPW-4	.00456667	.00275694	.115	-.0012255	.0103588
	CDW at pH7 for BKW-5	CDW at pH6.5 for BKW-5	-.00213333	.00275694	.449	-.0079255	.0036588
		CDW at pH6.5 for BKW-31	-.00353333	.00275694	.216	-.0093255	.0022588
		CDW at pH6.5 for BPW-4	-.00413333	.00275694	.151	-.0099255	.0016588
		CDW at pH7 for BKW-31	-.01110000*	.00275694	.001	-.0168921	-.0053079
		CDW at pH7 for BPW-4	.00063333	.00275694	.821	-.0051588	.0064255
		CDW at pH7.5 for BKW-5	.00023333	.00275694	.933	-.0055588	.0060255
		CDW at pH7.5 for BKW-31	-.01376667*	.00275694	.000	-.0195588	-.0079745
		CDW at pH7.5 for BPW-4	.00043333	.00275694	.877	-.0053588	.0062255
	CDW at pH7 for BKW-31	CDW at pH6.5 for BKW-5	.00896667*	.00275694	.004	.0031745	.0147588
		CDW at pH6.5 for BKW-31	.00756667*	.00275694	.013	.0017745	.0133588
		CDW at pH6.5 for BPW-4	.00696667*	.00275694	.021	.0011745	.0127588
		CDW at pH7 for BKW-5	.01110000*	.00275694	.001	.0053079	.0168921
		CDW at pH7 for BPW-4	.01173333*	.00275694	.000	.0059412	.0175255
		CDW at pH7.5 for BKW-5	.01133333*	.00275694	.001	.0055412	.0171255
		CDW at pH7.5 for BKW-31	-.00266667	.00275694	.346	-.0084588	.0031255
		CDW at pH7.5 for BPW-4	.01153333*	.00275694	.001	.0057412	.0173255
	CDW at pH7 for BPW-4	CDW at pH6.5 for BKW-5	-.00276667	.00275694	.329	-.0085588	.0030255
		CDW at pH6.5 for BKW-31	-.00416667	.00275694	.148	-.0099588	.0016255
		CDW at pH6.5 for BPW-4	-.00476667	.00275694	.101	-.0105588	.0010255
		CDW at pH7 for BKW-5	-.00063333	.00275694	.821	-.0064255	.0051588
		CDW at pH7 for BKW-31	-.01173333*	.00275694	.000	-.0175255	-.0059412
		CDW at pH7.5 for BKW-5	-.00040000	.00275694	.886	-.0061921	.0053921
		CDW at pH7.5 for BKW-31	-.01440000*	.00275694	.000	-.0201921	-.0086079
		CDW at pH7.5 for BPW-4	-.00020000	.00275694	.943	-.0059921	.0055921

	CDW at pH7.5 for BKW-5	CDW at pH6.5 for BKW-5	-.00236667	.00275694	.402	-.0081588	.0034255
		CDW at pH6.5 for BKW-31	-.00376667	.00275694	.189	-.0095588	.0020255
		CDW at pH6.5 for BPW-4	-.00436667	.00275694	.131	-.0101588	.0014255
		CDW at pH7 for BKW-5	-.00023333	.00275694	.933	-.0060255	.0055588
		CDW at pH7 for BKW-31	-.01133333*	.00275694	.001	-.0171255	-.0055412
		CDW at pH7 for BPW-4	.00040000	.00275694	.886	-.0053921	.0061921
		CDW at pH7.5 for BKW-31	-.01400000*	.00275694	.000	-.0197921	-.0082079
		CDW at pH7.5 for BPW-4	.00020000	.00275694	.943	-.0055921	.0059921
	CDW at pH7.5 for BKW-31	CDW at pH6.5 for BKW-5	.01163333*	.00275694	.001	.0058412	.0174255
		CDW at pH6.5 for BKW-31	.01023333*	.00275694	.002	.0044412	.0160255
		CDW at pH6.5 for BPW-4	.00963333*	.00275694	.003	.0038412	.0154255
		CDW at pH7 for BKW-5	.01376667*	.00275694	.000	.0079745	.0195588
		CDW at pH7 for BKW-31	.00266667	.00275694	.346	-.0031255	.0084588
		CDW at pH7 for BPW-4	.01440000*	.00275694	.000	.0086079	.0201921
		CDW at pH7.5 for BKW-5	.01400000*	.00275694	.000	.0082079	.0197921
		CDW at pH7.5 for BPW-4	.01420000*	.00275694	.000	.0084079	.0199921
	CDW at pH7.5 for BPW-4	CDW at pH6.5 for BKW-5	-.00256667	.00275694	.364	-.0083588	.0032255
		CDW at pH6.5 for BKW-31	-.00396667	.00275694	.167	-.0097588	.0018255
		CDW at pH6.5 for BPW-4	-.00456667	.00275694	.115	-.0103588	.0012255
		CDW at pH7 for BKW-5	-.00043333	.00275694	.877	-.0062255	.0053588
		CDW at pH7 for BKW-31	-.01153333*	.00275694	.001	-.0173255	-.0057412
		CDW at pH7 for BPW-4	.00020000	.00275694	.943	-.0055921	.0059921
		CDW at pH7.5 for BKW-5	-.00020000	.00275694	.943	-.0059921	.0055921
		CDW at pH7.5 for BKW-31	-.01420000*	.00275694	.000	-.0199921	-.0084079
*. The mean difference is significant at the 0.05 level.							

Table 9 : Significant variation between variations temperature against CDW of certain isolate obtained from the mid-gut of E. foetida at LSD and 95% confidence interval for post hoc tests with Multiple Comparisons

Multiple Comparisons							
LSD							
Dependent Variable	(I) coding	(J) coding	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
CDW	CDW at 25oC for BKW-5	CDW at 25oC for BKW-31	-.0021000	.0010077	.052	-.004217	.000017
		CDW at 25oC for BPW-4	.0003000	.0010077	.769	-.001817	.002417
		CDW at 30oC for BKW-5	-.0669333*	.0010077	.000	-.069051	-.064816
		CDW at 30oC for BKW-31	-.0022667*	.0010077	.037	-.004384	-.000149
		CDW at 30oC for BPW-4	-.0020333	.0010077	.059	-.004151	.000084
		CDW at 37oC for BKW-5	.0028667*	.0010077	.011	.000749	.004984
		CDW at 37oC for BKW-31	.0021333*	.0010077	.048	.000016	.004251
		CDW at 37oC for BPW-4	.0022333*	.0010077	.040	.000116	.004351
	CDW at 25oC for BKW-31	CDW at 25oC for BKW-5	.0021000	.0010077	.052	-.000017	.004217
		CDW at 25oC for BPW-4	.0024000*	.0010077	.028	.000283	.004517
		CDW at 30oC for BKW-5	-.0648333*	.0010077	.000	-.066951	-.062716
		CDW at 30oC for BKW-31	-.0001667	.0010077	.870	-.002284	.001951
		CDW at 30oC for BPW-4	.0000667	.0010077	.948	-.002051	.002184
		CDW at 37oC for BKW-5	.0049667*	.0010077	.000	.002849	.007084
		CDW at 37oC for BKW-31	.0042333*	.0010077	.001	.002116	.006351
		CDW at 37oC for BPW-4	.0043333*	.0010077	.000	.002216	.006451
	CDW at 25oC for BPW-4	CDW at 25oC for BKW-5	-.0003000	.0010077	.769	-.002417	.001817
		CDW at 25oC for BKW-31	-.0024000*	.0010077	.028	-.004517	-.000283
		CDW at 30oC for BKW-5	-.0672333*	.0010077	.000	-.069351	-.065116
		CDW at 30oC for BKW-31	-.0025667*	.0010077	.020	-.004684	-.000449

		CDW at 30oC for BPW-4	-.0023333*	.0010077	.033	-.004451	-.000216
		CDW at 37oC for BKW-5	.0025667*	.0010077	.020	.000449	.004684
		CDW at 37oC for BKW-31	.0018333	.0010077	.086	-.000284	.003951
		CDW at 37oC for BPW-4	.0019333	.0010077	.071	-.000184	.004051
	CDW at 30oC for BKW-5	CDW at 25oC for BKW-5	.0669333*	.0010077	.000	.064816	.069051
		CDW at 25oC for BKW-31	.0648333*	.0010077	.000	.062716	.066951
		CDW at 25oC for BPW-4	.0672333*	.0010077	.000	.065116	.069351
		CDW at 30oC for BKW-31	.0646667*	.0010077	.000	.062549	.066784
		CDW at 30oC for BPW-4	.0649000*	.0010077	.000	.062783	.067017
		CDW at 37oC for BKW-5	.0698000*	.0010077	.000	.067683	.071917
		CDW at 37oC for BKW-31	.0690667*	.0010077	.000	.066949	.071184
		CDW at 37oC for BPW-4	.0691667*	.0010077	.000	.067049	.071284
	CDW at 30oC for BKW-31	CDW at 25oC for BKW-5	.0022667*	.0010077	.037	.000149	.004384
		CDW at 25oC for BKW-31	.0001667	.0010077	.870	-.001951	.002284
		CDW at 25oC for BPW-4	.0025667*	.0010077	.020	.000449	.004684
		CDW at 30oC for BKW-5	-.0646667*	.0010077	.000	-.066784	-.062549
		CDW at 30oC for BPW-4	.0002333	.0010077	.820	-.001884	.002351
		CDW at 37oC for BKW-5	.0051333*	.0010077	.000	.003016	.007251
		CDW at 37oC for BKW-31	.0044000*	.0010077	.000	.002283	.006517
		CDW at 37oC for BPW-4	.0045000*	.0010077	.000	.002383	.006617
	CDW at 30oC for BPW-4	CDW at 25oC for BKW-5	.0020333	.0010077	.059	-.000084	.004151
		CDW at 25oC for BKW-31	-.0000667	.0010077	.948	-.002184	.002051
		CDW at 25oC for BPW-4	.0023333*	.0010077	.033	.000216	.004451
		CDW at 30oC for BKW-5	-.0649000*	.0010077	.000	-.067017	-.062783
		CDW at 30oC for BKW-31	-.0002333	.0010077	.820	-.002351	.001884
		CDW at 37oC for BKW-5	.0049000*	.0010077	.000	.002783	.007017
		CDW at 37oC for BKW-31	.0041667*	.0010077	.001	.002049	.006284
		CDW at 37oC for BPW-4	.0042667*	.0010077	.000	.002149	.006384

	CDW at 37oC for BKW-5	CDW at 25oC for BKW-5	-.0028667*	.0010077	.011	-.004984	-.000749
		CDW at 25oC for BKW-31	-.0049667*	.0010077	.000	-.007084	-.002849
		CDW at 25oC for BPW-4	-.0025667*	.0010077	.020	-.004684	-.000449
		CDW at 30oC for BKW-5	-.0698000*	.0010077	.000	-.071917	-.067683
		CDW at 30oC for BKW-31	-.0051333*	.0010077	.000	-.007251	-.003016
		CDW at 30oC for BPW-4	-.0049000*	.0010077	.000	-.007017	-.002783
		CDW at 37oC for BKW-31	-.0007333	.0010077	.476	-.002851	.001384
		CDW at 37oC for BPW-4	-.0006333	.0010077	.538	-.002751	.001484
	CDW at 37oC for BKW-31	CDW at 25oC for BKW-5	-.0021333*	.0010077	.048	-.004251	-.000016
		CDW at 25oC for BKW-31	-.0042333*	.0010077	.001	-.006351	-.002116
		CDW at 25oC for BPW-4	-.0018333	.0010077	.086	-.003951	.000284
		CDW at 30oC for BKW-5	-.0690667*	.0010077	.000	-.071184	-.066949
		CDW at 30oC for BKW-31	-.0044000*	.0010077	.000	-.006517	-.002283
		CDW at 30oC for BPW-4	-.0041667*	.0010077	.001	-.006284	-.002049
		CDW at 37oC for BKW-5	.0007333	.0010077	.476	-.001384	.002851
		CDW at 37oC for BPW-4	.0001000	.0010077	.922	-.002017	.002217
	CDW at 37oC for BPW-4	CDW at 25oC for BKW-5	-.0022333*	.0010077	.040	-.004351	-.000116
		CDW at 25oC for BKW-31	-.0043333*	.0010077	.000	-.006451	-.002216
		CDW at 25oC for BPW-4	-.0019333	.0010077	.071	-.004051	.000184
		CDW at 30oC for BKW-5	-.0691667*	.0010077	.000	-.071284	-.067049
		CDW at 30oC for BKW-31	-.0045000*	.0010077	.000	-.006617	-.002383
		CDW at 30oC for BPW-4	-.0042667*	.0010077	.000	-.006384	-.002149
		CDW at 37oC for BKW-5	.0006333	.0010077	.538	-.001484	.002751
		CDW at 37oC for BKW-31	-.0001000	.0010077	.922	-.002217	.002017
*. The mean difference is significant at the 0.05 level.							

Table 10: Significant variation between variations for B. subtilis BKW-5**against various carbon and nitrogen source at LSD and 95% confidence interval for post hoc tests with Multiple Comparisons**

Multiple Comparisons						
Dependent Variable: CDW LSD						
(I) CODE	(J) CODE	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
CDW (g/L) for BKW-5 against Glucose	CDW (g/L) for BKW-5 against Maltose	.0025000*	.0008398	.012	.000670	.004330
	CDW (g/L) for BKW-5 against Mannitol	.0019667*	.0008398	.037	.000137	.003796
	CDW (g/L) for BKW-5 against Peptone	.0005333	.0008398	.537	-.001296	.002363
	CDW (g/L) for BKW-5 against KNO3	.0005333	.0008398	.537	-.001296	.002363
	CDW (g/L) for BKW-5 against YE	.0009000	.0008398	.305	-.000930	.002730
CDW (g/L) for BKW-5 against Maltose	CDW (g/L) for BKW-5 against Glucose	-.0025000*	.0008398	.012	-.004330	-.000670
	CDW (g/L) for BKW-5 against Mannitol	-.0005333	.0008398	.537	-.002363	.001296
	CDW (g/L) for BKW-5 against Peptone	-.0019667*	.0008398	.037	-.003796	-.000137
	CDW (g/L) for BKW-5 against KNO3	-.0019667*	.0008398	.037	-.003796	-.000137
	CDW (g/L) for BKW-5 against YE	-.0016000	.0008398	.081	-.003430	.000230
CDW (g/L) for BKW-5 against Mannitol	CDW (g/L) for BKW-5 against Glucose	-.0019667*	.0008398	.037	-.003796	-.000137
	CDW (g/L) for BKW-5 against Maltose	.0005333	.0008398	.537	-.001296	.002363
	CDW (g/L) for BKW-5 against Peptone	-.0014333	.0008398	.114	-.003263	.000396
	CDW (g/L) for BKW-5 against KNO3	-.0014333	.0008398	.114	-.003263	.000396
	CDW (g/L) for BKW-5 against YE	-.0010667	.0008398	.228	-.002896	.000763
CDW (g/L) for BKW-5 against Peptone	CDW (g/L) for BKW-5 against Glucose	-.0005333	.0008398	.537	-.002363	.001296
	CDW (g/L) for BKW-5 against Maltose	.0019667*	.0008398	.037	.000137	.003796
	CDW (g/L) for BKW-5 against Mannitol	.0014333	.0008398	.114	-.000396	.003263
	CDW (g/L) for BKW-5 against KNO3	0E-7	.0008398	1.000	-.001830	.001830

	CDW (g/L) for BKW-5 against YE	.0003667	.0008398	.670	-.001463	.002196
CDW (g/L) for BKW-5 against KNO3	CDW (g/L) for BKW-5 against Glucose	-.0005333	.0008398	.537	-.002363	.001296
	CDW (g/L) for BKW-5 against Maltose	.0019667*	.0008398	.037	.000137	.003796
	CDW (g/L) for BKW-5 against Mannitol	.0014333	.0008398	.114	-.000396	.003263
	CDW (g/L) for BKW-5 against Peptone	0E-7	.0008398	1.000	-.001830	.001830
	CDW (g/L) for BKW-5 against YE	.0003667	.0008398	.670	-.001463	.002196
CDW (g/L) for BKW-5 against YE	CDW (g/L) for BKW-5 against Glucose	-.0009000	.0008398	.305	-.002730	.000930
	CDW (g/L) for BKW-5 against Maltose	.0016000	.0008398	.081	-.000230	.003430
	CDW (g/L) for BKW-5 against Mannitol	.0010667	.0008398	.228	-.000763	.002896
	CDW (g/L) for BKW-5 against Peptone	-.0003667	.0008398	.670	-.002196	.001463
	CDW (g/L) for BKW-5 against KNO3	-.0003667	.0008398	.670	-.002196	.001463
*. The mean difference is significant at the 0.05 level.						

Table 11: Significant variation for B. flexus BKW-31 against various carbon and nitrogen source at LSD and 95% confidence interval for post hoc tests with Multiple Comparisons

Multiple Comparisons						
Dependent Variable: CDW LSD						
(I) Code	(J) Code	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
CDW (g/L) for BKW-31 for Glucose	CDW (g/L) for BKW-31 for Maltose	.0026667	.0012255	.050	-.000003	.005337
	CDW (g/L) for BKW-31 for Mannitol	.0018000	.0012255	.168	-.000870	.004470
	CDW (g/L) for BKW-31 for Peptone	-.0070000*	.0012255	.000	-.009670	-.004330
	CDW (g/L) for BKW-31 for KNO3	.0018667	.0012255	.154	-.000803	.004537
	CDW (g/L) for BKW-31 for YE	-.0000667	.0012255	.958	-.002737	.002603
CDW (g/L) for BKW-31 for Maltose	CDW (g/L) for BKW-31 for Glucose	-.0026667	.0012255	.050	-.005337	.000003
	CDW (g/L) for BKW-31 for Mannitol	-.0008667	.0012255	.493	-.003537	.001803
	CDW (g/L) for BKW-31 for Peptone	-.0096667*	.0012255	.000	-.012337	-.006997
	CDW (g/L) for BKW-31 for KNO3	-.0008000	.0012255	.526	-.003470	.001870
	CDW (g/L) for BKW-31 for YE	-.0027333*	.0012255	.046	-.005403	-.000063
CDW (g/L) for BKW-31 for Mannitol	CDW (g/L) for BKW-31 for Glucose	-.0018000	.0012255	.168	-.004470	.000870
	CDW (g/L) for BKW-31 for Maltose	.0008667	.0012255	.493	-.001803	.003537
	CDW (g/L) for BKW-31 for Peptone	-.0088000*	.0012255	.000	-.011470	-.006130
	CDW (g/L) for BKW-31 for KNO3	.0000667	.0012255	.958	-.002603	.002737
	CDW (g/L) for BKW-31 for YE	-.0018667	.0012255	.154	-.004537	.000803
CDW (g/L) for BKW-31 for Peptone	CDW (g/L) for BKW-31 for Glucose	.0070000*	.0012255	.000	.004330	.009670
	CDW (g/L) for BKW-31 for Maltose	.0096667*	.0012255	.000	.006997	.012337
	CDW (g/L) for BKW-31 for Mannitol	.0088000*	.0012255	.000	.006130	.011470
	CDW (g/L) for BKW-31 for KNO3	.0088667*	.0012255	.000	.006197	.011537
	CDW (g/L) for BKW-31 for YE	.0069333*	.0012255	.000	.004263	.009603
CDW (g/L) for BKW-31 for KNO3	CDW (g/L) for BKW-31 for Glucose	-.0018667	.0012255	.154	-.004537	.000803
	CDW (g/L) for BKW-31 for Maltose	.0008000	.0012255	.526	-.001870	.003470

	CDW (g/L) for BKW-31 for Mannitol	-.0000667	.0012255	.958	-.002737	.002603
	CDW (g/L) for BKW-31 for Peptone	-.0088667*	.0012255	.000	-.011537	-.006197
	CDW (g/L) for BKW-31 for YE	-.0019333	.0012255	.141	-.004603	.000737
CDW (g/L) for BKW-31 for YE	CDW (g/L) for BKW-31 for Glucose	.0000667	.0012255	.958	-.002603	.002737
	CDW (g/L) for BKW-31 for Maltose	.0027333*	.0012255	.046	.000063	.005403
	CDW (g/L) for BKW-31 for Mannitol	.0018667	.0012255	.154	-.000803	.004537
	CDW (g/L) for BKW-31 for Peptone	-.0069333*	.0012255	.000	-.009603	-.004263
	CDW (g/L) for BKW-31 for KNO3	.0019333	.0012255	.141	-.000737	.004603
*. The mean difference is significant at the 0.05 level.						

Table 12: Significant variation between variations for B. cereus BKW-4 against various carbon and nitrogen source at LSD and 95% confidence interval for post hoc tests with Multiple Comparisons

Multiple Comparisons						
Dependent Variable: CDW LSD						
(I) Code	(J) Code	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
CDW (g/L) for BPW-4 against Glucose	CDW (g/L) for BPW-4 against Maltose	.0027333*	.0005277	.000	.001583	.003883
	CDW (g/L) for BPW-4 against Mannitol	.0023667*	.0005277	.001	.001217	.003517
	CDW (g/L) for BPW-4 against Peptone	-.0018000*	.0005277	.005	-.002950	-.000650
	CDW (g/L) for BPW-4 against KNO3	-.0011667*	.0005277	.047	-.002317	-.000017
	CDW (g/L) for BPW-4 against YE	-.0007000	.0005277	.209	-.001850	.000450
CDW (g/L) for BPW-4 against Maltose	CDW (g/L) for BPW-4 against Glucose	-.0027333*	.0005277	.000	-.003883	-.001583
	CDW (g/L) for BPW-4 against Mannitol	-.0003667	.0005277	.500	-.001517	.000783
	CDW (g/L) for BPW-4 against Peptone	-.0045333*	.0005277	.000	-.005683	-.003383
	CDW (g/L) for BPW-4 against KNO3	-.0039000*	.0005277	.000	-.005050	-.002750
	CDW (g/L) for BPW-4 against YE	-.0034333*	.0005277	.000	-.004583	-.002283
CDW (g/L) for BPW-4 against Mannitol	CDW (g/L) for BPW-4 against Glucose	-.0023667*	.0005277	.001	-.003517	-.001217
	CDW (g/L) for BPW-4 against Maltose	.0003667	.0005277	.500	-.000783	.001517
	CDW (g/L) for BPW-4 against Peptone	-.0041667*	.0005277	.000	-.005317	-.003017
	CDW (g/L) for BPW-4 against KNO3	-.0035333*	.0005277	.000	-.004683	-.002383
	CDW (g/L) for BPW-4 against YE	-.0030667*	.0005277	.000	-.004217	-.001917
CDW (g/L) for BPW-4 against Peptone	CDW (g/L) for BPW-4 against Glucose	.0018000*	.0005277	.005	.000650	.002950
	CDW (g/L) for BPW-4 against Maltose	.0045333*	.0005277	.000	.003383	.005683
	CDW (g/L) for BPW-4 against Mannitol	.0041667*	.0005277	.000	.003017	.005317

	CDW (g/L) for BPW-4 against KNO3	.0006333	.0005277	.253	-.000517	.001783
	CDW (g/L) for BPW-4 against YE	.0011000	.0005277	.059	-.000050	.002250
CDW (g/L) for BPW-4 against KNO3	CDW (g/L) for BPW-4 against Glucose	.0011667*	.0005277	.047	.000017	.002317
	CDW (g/L) for BPW-4 against Maltose	.0039000*	.0005277	.000	.002750	.005050
	CDW (g/L) for BPW-4 against Mannitol	.0035333*	.0005277	.000	.002383	.004683
	CDW (g/L) for BPW-4 against Peptone	-.0006333	.0005277	.253	-.001783	.000517
	CDW (g/L) for BPW-4 against YE	.0004667	.0005277	.394	-.000683	.001617
CDW (g/L) for BPW-4 against YE	CDW (g/L) for BPW-4 against Glucose	.0007000	.0005277	.209	-.000450	.001850
	CDW (g/L) for BPW-4 against Maltose	.0034333*	.0005277	.000	.002283	.004583
	CDW (g/L) for BPW-4 against Mannitol	.0030667*	.0005277	.000	.001917	.004217
	CDW (g/L) for BPW-4 against Peptone	-.0011000	.0005277	.059	-.002250	.000050
	CDW (g/L) for BPW-4 against KNO3	-.0004667	.0005277	.394	-.001617	.000683
*. The mean difference is significant at the 0.05 level.						