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Cellulase Sources in the Eudrilid Earthworm, *Eudrilus Eugeniae*

[1]Aladesida, A.A., [2]Owa, S.O., [1]Dedeke, G.A., [3]Osho, B.A. and [4]Adewoyin, O.A.

[1]Dept. of Biological Sciences, Federal University of Agriculture, Abeokuta, Nigeria

[2]Dept. of Biological Sciences, Landmark University, Omu-Aran, Nigeria

[3]Formerly of the Dept. of Biological Sciences, OlabisiOnabanjo University, Ago-Iwoye

[4]Department of Zoology, University of Ilorin, Kwara State, Nigeria

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Abstract: Earthworms have been known to breakdown leaf litter to release the nutrients laden in the leaves. Their ability to breakdown leaf litter implies that they have a source of cellulose. The present work looked at these sources to establish if they were exogenous or endogenous in origin. Earthworm samples of the species *Eudriluseugeniae*were fed with tissue paper as cellulose source. The resulting faecal droppings were subjected to various carbohydrate tests to confirm the final products. Some of the worms were defaunated by treating with antibiotics to ensure the cellulose source was exogenous. Survival tests were also carried out on the faunated and defaunated earthworms. The results showed by Molisch's test the presence of carbohydrate in both fresh and egested tissue paper; while Benedict test and Barfoed's test indicate that the ingested tissue paper was digested to monosaccharide level. Seliwanoff's test also confirmed that the breakdown product was the monosaccharide fructose. Result of the survival test showed a significantly higher survival rate in the faunated than the defaunated earthworms. The results also showed that cellulose is of both exogenous and endogenous source to the earthworms. It is thus proposed that in plant litter treatments introduction of earthworms should be encouraged above merely introducing microbes as the gut of the worms is here shown to support microbial activity.

Keywords: Earthworms, Cellulose, faunated, defaunated, exogenous and endogenous

INTRODUCTION

Earthworms are ubiquitous, being among the most widely distributed invertebrates occurring over most of the earth, and preferring moist soil rich in calcium and organic matter. Madge[1] noted that earthworms literally eat their way through soil, and the ingested earth is passed through the digestive tract and deposited at the surface in small mounds or 'castings'. That earthworm activity play important roles in increasing soil fertility and productivity is universally acknowledged [2-9]. Earthworms are known to increase soil porosity and soil aeration, bringing about decomposition of leaf and other litters, improve soil nitration and nutrient by excretion of nitrogenous wastes as detoxification of some waste materials in soil[10-11].

Another major area in earthworm research is their use in biodegradation of domestic and agricultural wastes by a process known as vermicomposting [12]. The earthworms act on organic waste through a combination of efforts, [10, 13] which involve the enteric microbes in the earthworm gut and secretions by the worms. The mucus coated egested material, known as vermicast which is richer in nutrient and useful microbes than the parent soil has been shown to have tremendous effects on plant growth [9,13,14]. Owa*et al*. [14] and Dynes[15] also noted that this egested material, i.e. vermicast is rich in auxins and cytocinins which are plant growth hormones.

These enteric microbes play an important part in these activities of the earthworms. These microbes are ingested from the soil as earthworms feed[16]. Some such microbes are not just passers-by but permanent dwellers in definite regions of the earthworm gut[17]. The microbes are multiplied as they transit earthworm gut and are dispersed as the earthworms move around[18-19].

The present study was carried out to establish the sources of cellulase in the earthworm species *Eudriluseugeniae*Kinberg, 1866, a worm being considered in some vermicomposting research. The broad aim is to be able to establish the best approach to the use of the worms and microbes that may be found to be involved in the breakdown of cellulose in the leaves fed on by the worms. This it is believe will guide in determining the benefit of using the worms directly over using the microbes directly on the litter.

MATERIALS AND METHODS

Tests for carbohydrate carried out on egested matters of earthworms fed on cellulose: Tissue paper was used as the source of cellulose in this experiment. It was initially tested on iodine solution for starch, and was fed to some earthworms (all samples used were *Eudriluseugeniae*Kinberg, 1866). The faecal droppings of the earthworms fed tissue paper were tested for carbohydrates. Control tests were carried out on tissue paper, which was not fed to earthworm. The tests were as follows.

Molisch's test, which is general for all types of carbohydrates, was carried out by adding two drops of 1% alcoholic naphthol to 1ml of the suspension containing the egested matter. Then concentrated H_2SO_4 was allowed to run gently down the side of the test tube. The violet ring formed at the interphase indicated the presence of carbohydrates.

Benedict's test, used for detecting disaccharides, was carried out to determine whether the tissue paper meal was digested to disaccharide. 2 ml of Benedict's solution was transferred to 5 drops of the test solution in a boiling tube, and heat was applied in a water bath for 2-3 minutes. A red colour after heating indicated the presence of reducing disaccharides.

Barfoed's test, for determining whether the test solution contains a monosaccharide or disaccharide, was carried out by adding 1 ml of the test solution to 2 ml of Barfoed's reagent, boiled in water bath for 1 min., and allowed to stand. An orange to red colour indicated the presence of monosaccharide.

Seliwanoff's test, used to distinguish between the monosaccharides (fructose and glucose), was carried out by adding three drops of the solution to 3 ml of Seliwanoff's reagent. This was then heated in a water bath for one minute. An orange precipitate indicated the presence of fructose.

All the above tests were carried out on the test solution made by dispensing 0.5 g of the egested matter in 20 ml of distilled water.

Iodine test for starch on egested matters: The egested matters were tested for starch by flooding them with iodine. Blue-black colour would indicate positive i.e. presence of starch.

Survival test on earthworms: Ten faunated and 10 defaunated earthworms were fed continuously on tissue paper cellulose as the only carbon source. Their survival was monitored until they died.

Test to show whether earthworms produce cellulose endogenously or exogenously: Ten earthworms were fed for 48 hours on cellulose to which streptomycin had been added as an antibacterial to eliminate enteric bacteria of the earthworms, after which the earthworms were fed on cellulose alone. Similarly, another set of ten earthworms were fed for 48 hours on cellulose to which the antimycotic lactic acid had been added after which they were fed on cellulose alone.

RESULTS

From the tests for carbohydrates, disaccharides and monosaccharides in the tissue paper and florated and defaunated earthworms, Molisch's test indicated the presence of carbohydrate in both fresh and egested tissue paper. The negative results of Benedict test coupled with the positive result of Barfoed's test indicate that the ingested tissue paper had digested beyond disaccharide to monosaccharide level. Seliwanoff's test also confirmed that the breakdown product is the monosaccharide fructose (**Table 1**).

Table-1: Reactions of tissue paper and faecal pellets from faunated and defaunated Earthworms to different carbohydrate tests

Tests	Tissue paper	Egested matter by faunated earthworms	Egested matter by defaunated earthworms
Molisch	Positive	Positive	Positive
Benedict	Negative	Negative	Negative
Barfoed	Negative	Positive	Positive
Seliwanoff	Negative	Positive	Positive

This result indicates that carbohydrate is present in both fresh and egested tissue paper. The positive result of Barfoed and Seliwanoff tests on the egested tissue paper from both faunated and defaunated earthworms indicate that despite the voiding of the enteric microflora in the defaunated earthworms, they were still able to break down cellulose to monosaccharides and specifically to fructose as much as the faunated earthworms (Table 1). This shows that cellulose produced in these earthworms is of both endogenous and exogenous source. The iodine test on the egested materials was negative, showing that the cellulose was not converted to starch.

Result of survival test of faunated and defaunated earthworms fed only on cellulose: The survival rate of the earthworms as shown in **Table 2** presents a gradual decline in the number of individuals which survived as the number of days of the experiment increased. By the 36th days all the defaunated earthworms were dead, while it took an additional 9 days before the last faunated worm died.

A comparison of the time it took to produce faecal pellet by the faunated and defaunated earthworms showed that it took a longer time (35 days) for defaunated earthworms to produce 5g of faecal pellets while the faunated worms took 11days to produce the same weight of faecal pellets (**Table 3**). This confirms that the contribution of the gut microflora to cellulase production in the earthworm is of utmost importance in the feeding activity of the worms.

Table-2: Survival of earthworms fed only on cellulose

Time (days)	No of faunated earthworms still surviving	No of defaunated earthworms still surviving
0	10	10
4	10	10
8	10	6
12	9	5
16	9	4
20	7	4
24	6	3
28	6	3
32	5	2
36	3	0

Table- 3: Rate of cellulose degradation by defaunated and faunated earthworms

Earthworm treatment	Time (days) taken to produce 5g of faecal pellet
Defaunated	35
Faunated	11

DISCUSSION AND RECOMMENDATIONS

It has been estimated that the weight of leaves that falls annually in woodland varies from as little as 500 kg per ha per year in alpine and arctic forests to as much as 2,500 - 3,500 kg per year in temperate forests and to as much as 5,500-15,000 kg per ha per year in tropical forests[20]. Satchell,[21] calculated that if a temperate deciduous woodland has a leaf fall of 3,000 kg per ha per year and if earthworms consume 27 mg per g of leaf litter per day, which is a reasonable average expectation, then they would consume the annual leaf fall in about three months. Madge,[22] calculated that in tropical forests in Nigeria, the litter fall was three or four times as much as in the temperate forests and suggested that earthworms were the most important animal in fragmenting and incorporating leaves into the soil. Results from this study shows that earthworms are able to degrade cellulose, which indicates that they are able to produce cellulase. This is of importance in the consumption of leaf litter in the soil because these litters have cellulose cell walls which any organism that feeds on them must degrade. The consumption of these leaf litters is important because by it earthworms help in recycling soil nutrients.

The result also indicated that the cellulose produced is both exogenous and endogenous. This must have added to the effectiveness to which the earthworms degrade the cellulose.

The potential of earthworms in waste paper recycling: The present result, which shows that earthworms can degrade cellulose when fed on carbon source alone, suggests that earthworms can be used in degrading paper wastes and leaf litter instead of the usual burning of such wastes. Using earthworms for this purpose should be preferred because the smoke from the burning constitutes one of the major sources atmospheric pollution in the tropics. The heat generated during the burning may be of harmful effects to the soil microflora and fauna. In addition, the ashes to which this waste paper is reduced are less useful because they are soon washed off by rain water. Therefore, using earthworms to degrade paper wastes and leaf litters would be economical, less laborious and of better results than burning.

Earthworms in humification of litter: Earthworms also help in the decomposition of organic matters, the final process of which is known as humification. This process is simply the breaking down of large particles of organic matter into complex amorphous colloid containing phenolic materials. Only about one quarter of the fresh organic matter becomes converted to humus. Much of the humification process is due to smaller soil organisms like micro-organisms mites, springtails and other arthropods, but is also accelerated by the passage of the organic material through the guts of earthworms feeding on decomposed organic matter together with mineral soil. Probably some of the final stages of humification are due to the intestinal miroflora in the earthworm's gut because most of the evidence indicates that the processes of humification are caused more by the microflora than by fauna.

Importance of enteric microbes of the earthworms: Result from the present study also shows that the rate at which the earthworms degrade the cellulose without their intestinal microflora was about 31% slower than when degradation was done with the aid of the intestinal microflora. Thus, with the aid of the intestinal microflora, the degradation of cellulose will be very fast since the passing of these litters through the earthworm gut accelerates their rate of degradation. This suggests that introducing the microbes directly may not be as effective for cellulose litter degradation as making use of the earthworms infested with the microbes. Previous works by Owa*et al*.[9] and Mba,[10] confirmed that there is microbial multiplication in the earthworm gut, which may explain the high difference in the rate of production of cast between the faunated and defaunated earthworms in this study.

From results obtained, earthworms can be said to be very effective in inoculating the soil with microbes. A soil is said to be good for planting when there are enough nutrients and humus. The process of humification depends on microbes, which are inoculated into the soil by earthworms through their casts.

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***Corresponding Author: Aladesida;** Dept. of Biological Sciences, Federal University of Agriculture, Abeokuta, Nigeria