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Enteric bacteria and fungi of the Eudrilid earthworm *Libyodrilus violaceus*

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The identity and multiplication of bacteria and fungi (yeasts and mould) as they pass along the alimentary tract of the earthworm *Libyodrilus violaceus* have been studied. The bacteria isolated included *Acinetobacter* sp., *Alcaligans faecalis, Bacillus brevis, Bacillus ceveus, Bacillus lalerosporus, Bacillus lichenoform, Bacillus maceraus, Bacillus* sp., *Corynebacterium* sp., *Enterobacter cloacae, Erwinia salicie, Flavobacterium aquartile, Flavobacterium* sp., *Klebsiella* sp., *Micrococcus inteus, Micrococcus kristinae, Micrococcus varians, Proteus myxofasciens, Proteus rennevi, Proteus vulgaris* and *Pseudomonas* sp. Whereas *P. vulgaris* is a normal harmless inhabitant of the human intestine where it assist with digestion, it sometimes becomes pathogenic causing urinary tract infection. For now there is no information on if it undergoes similar change in the earthworms and if such a potential risk is transmissible to man. The fungi isolated included the following yeasts: *Saccharomycos cerevisiae, Rhodoturula graminis, Saccharomycos* sp., *Candida valida, Geotrichium niger;* and the following moulds: *Aspergillus niger, Aspergillus fumigatus, Fusarium* sp., *Rhizopus* sp. It is noteworthy that none of the fungi has the ability to digest melobiose, a disaccharide formed by an alpha linkage between galactose and glucose. Microbial counts increases along the alimentary track from eosophagus to rectum. Most of the microbes flourish best in an alimentary track region than in others. Thus, they tend to colonize different regions and thus minimize competition.

Key words: Eudrilidae, earthworm microbiology, agricultural microbiology, earthworm zoonosis, earthworm-microbe mutualism, soil microbiology.

INTRODUCTION

The importance of earthworms is too well known to be described in any details here. They are involved in many aspects of the soil: Pedogenesis (Feller et al., 2003; Morgan et al., 2002; Eric et al., 2003); soil aggregate formation (Sharpley et al., 1979); soil aeration (Owa et al., 2002; Edwards and Heath 1963); humus formation (Feller et al., 2003); litter recycling; etc.

Much of these functions depend on their symbiotic association with enteric microbes that colonize the buccal cavity, pharynx, gizzard, intestine and rectum (Lynch and Poole 1979). From these enteric microhabitats the microbes are known to be involved with such functions as digestion of cellulose-containing organic matters such as leaf, and in chemical weathering of rocks (Morgan et al.,

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2002).The nature of the microbes has been investigated. For example, Pedersen and Hendriksen (1993) and Hendriksen (1995) show that non-spore forming, Gram-negative soil bacteria can survive passage through the gut of earthworms. The ability of the microbes to survive the earthworm enteric conditions is also very important and it could serve as a biological factory sieve. For example, it has been reported that many plant-pathogenic microbes die during passage through worm gut (Marks and Cooper 1977). It is even believed that these could serve as ecological sieve to eliminate or reduce the proportion of plant-pathogenic microbes in the soil, and therefore act as pathogenicide (Singleton et al., 2003).

Another area of active interest is the multiplication effect as microbes pass through the gut of earthworms (Hendriksen, 1995; Singleton et al., 2003; Owa et al., 2009).

The finding of butyric acid-forming bacteria of the clostridium type in the gut of some earthworms is yet another area earthworm interest. Their presence therefore facilitates the formation of the indo-acetic acid, an auxin, a plant growth hormone (Sigleton et al., 2003).

The existence of cellulose digesting microbes in the gut of earthworms facilitates the use of earthworm in leaf litter recycling, and waste paper management (Loquet and Vinceslas, 1987).

Because of the economic importance of these worm-microbe associations, it is important to study the situation in the tropical forests where: (a) the volume of leaf litter is higher than in any other part of the world; (b) due to high relative humidity, microbial population is very high, including those that are plant pathogenic and (c) the soil tends to be acidic and needs microbial activities to initiate soil acidity buffering actions.

The present study was therefore carried out to estimate the multiplicative gains in microbial count during passage through the gut of the earthworms *Libyodrilus violaceu* and iIdentify microbial species found along the gut of the earthworm.

MATERIALS AND METHODS

This study was carried out on the earthworm *L. violaceus* collected from a swampy soil around Olabisi Onabanjo University, Ago-Iwoye, Ogun State, Nigeria. The laboratory studies were carried out at the Federal Institute of Industrial Research (FIIRO), Oshodi, Lagos, Nigeria.

Sterilization/aseptic techniques of material and media

Aseptic methods were adopted. All glassware were sterilised in an oven at 160℃ or in an autoclave at a pressure of 1 atm, at 121℃ for 15 min. The media, prepared in conical flasks, and the distilled water, were similarly autoclave-sterilized. The dissecting materials, that is, the dissecting board, kits and the scissors were aseptically sterilized by wiping with cotton wool dipped in absolute methylated spirit or 95% alcohol. Before the experiment, the working bench and areas were sterilized by wiping with cotton wool dipped in absolute methylated spirit or 95% alcohol and a spirit lamp was lit up to ensure that the air in the vicinity of the working bench is free of contaminants. The inoculating loop was sterilized by flaming over a spirit lamp until red hot and then allowed to cool before use and sterile pipette was used just once for every experiment.

Coughing, talking and sneezing were avoided during the experiment to prevent contamination.

Earthworm sample collection

Using a spade, the earthworms were handpicked into containers and were transported to the laboratory where they were washed with clean sterile water. The worms were kept under refrigeration for three to four hours in order to kill them without causing any harm or alteration to the microbial gut content.

Preparation of media

The media used are nutrient broth (NB), nutrient agar (NA), potato dextrose agar (PDA), urease medium, and Simmon's citrate agar etc.

Dissection of samples

Worms were pinned down horizontally on the dissecting wood board with the dorsal part downward and the gizzard, intestine and rectum identified. Using sterile spatula, 0.5 g of the gut contents of the target regions were carefully transferred into 20 ml of sterile nutrient broth and incubated for ten days at room temperature.

Preparation of serial dilutions

Test tubes containing 9 ml of sterile distilled water was set up into tube racks. 1 ml of the sample (from the nutrient) broth was transferred with a sterile pipette into one of the tubes labelled 10^{-1} dilution and was shaken properly. By a similar procedure serial dilutions of 10^{-2} and 10^{-4} were prepared. The same procedure was carried out on PDA on order to compare the population of microbial growth.

Methods of inoculation

Two main inoculation methods involved were:

1. Pour plate technique: This involves the aseptic transfer of 0.1 ml aliquots of the diluted sample onto sterile Petri dishes. Cooled molten agar of Nutrient agar were poured at 42℃ for the isolation and enumeration of bacteria; Potato dextrose agar (PDA) for the isolation yeast and fungi. The plates were mixed clock wisely and anti-clock wisely to evenly distribute the inoculums in the medium solution. The plates were allowed to set.
2. Isolates were made by streaking. This involved the use of sterile wire loop to pick up a discrete colony of interest from the mixed culture plate, and using it to make distinct streaks on the surface of he sterile agar. Care was taken to flame the picking loop after each sterile streak.

Incubation

Inoculated plates for both bacteria and fungi were incubated in an inverted position on NA at 37℃ for a period between 24 and 48 h

Table 1. Bacterial count along the gut of the earthworm *L. violaceus:* Total viable counts on nutrient agar (cfu/ml × 10^{-7}).

Replicates	Worm one				Worm two				Worm three				N	Mean	SD
	1	2	3	4	1	2	3	4	1	2	3	4			
Gizzard	52	54	55	50	63	60	60	63	43	41	42	43	12	52.2	8.36
Intestine	59	60	58	59	68	66	64	64	60	62	61	62	12	61.92	3.06
Rectum	65	66	67	67	77	78	68	67	73	71	72	68	12	69.50	4.72

Table 2. Analysis of variance to show that the distribution of bacteria in the three regions of the gut is not uniform.

Dependent variable		Sum of Sq.	df	Mean Sq.	F	Sig.
Count of bacteria in N.A.	Between groups	1812.056	2	906.028	26.753	0.000
	Within groups	1117.583	33	33.866		
	Total	2929.639	35			

Table 3. Multiple comparisons to show that no two regions of the gut had the same bacterial count.

Dependent variable	(I) Organ	(J) Organ	Mean difference (I-J)	S.E.	Sig.
Count of bacteria in N.A.	Gizzard	Intestine	-9.75	2.38	0.000
		Rectum	-17.33	2.38	0.000
	Intestine	Gizzard	9.75	2.38	0.000
		Rectum	-7.58	2.38	0.003
	Rectum	Gizzard	17.33	2.38	0.000
		Intestine	7.58	2.38	0.003

for bacteria, 27°C for yeast and moulds for 5 days.

Identification of isolates

The isolates were identified using a number of characteristics. Their cultural and morphological features were of vital importance in this process and were thus observed. Motility tests as well as biochemical tests were carried out. These tests included Gram stain reactions, motility test carried out using Edwards and Wing motility test medium, catalase production, oxidase test, indole production, urease activity, gelatin hydrolysis, sugar fermentation and spore staining.

RESULTS

Table 1 shows that the bacterial counts at the gizzard, intestine and the rectum increases from the anterior to the posterior end of the gut. Table 2 shows that the differences in the earthworm populations between the three regions were statistically significant. LSD analysis (Table 3) shows that no two enteric regions have the same bacterial count.

Yeast counts in the three different regions of the earthworm gut are shown in Table 4. Anova shows that the distribution is not uniform in the three regions (Table 5). In general, the counts increase posteriad. The multiple comparisons in Table 6 show which regions have comparable and incomparable counts.

Table 7 shows the mold count in the three regions of the earthworm gut. The counts are not uniform as shown by an analysis of variance (Table 8); the counts tend to increase from the gizzard region to towards the rectum. The rectum hosts the largest mold biomass. Count differences between regions are shown in Table 9.

As shown in Table 10, twenty one species of bacteria belonging to eleven genera were isolated. The most versatile (that is, isolated in the three organs) were Pseudomonas sp. and *Corynebacterium* sp. The next, Bacillus brevis and *Proteus myxofasciens*, were recorded from only two of the organs. All others were isolated from only one organ.

The following were recorded only in the gizzard – *Corynebacterium* sp., *Flavobacterium* sp. *Enterobacter cloacae*, *Acinetobacter* sp., *Bacillus maceraus*, *Bacillus* sp. and *Proteus rennevi*. Isolate only from the intestine were: *Micrococcus kristinae*, *Proteus vulgaris*, *Erwinia salicie*, *Bacillus lichenoform, Bacillus lalerosporus*, *Bacillus ceveus*, *Micrococcus inteus* and *Alcaligans*

Table 4. Yeast counts along the gut of the earthworm *L. violaceus:* Total viable counts on potato dextrose agar (cfu/ml × 10^{-7}).

Replicates	Worm one				Worm two				Worm three				N	Mean	S.D.
	1	2	3	4	1	2	3	4	1	2	3	4			
Gizzard	2	2	3	3	3	2	3	3	2	2	1	1	12	2.2500	0.7538
Intestine	3	3	2	3	ND	ND	ND	ND	1	2	1	1	8	2.0000	0.9258
Rectum	3	2	2	2	7	7	5	6	4	4	3	4	12	4.1667	1.7495

Table 5. Analysis of variance to show that the distribution of yeast in the three regions of the gut is not uniform.

Dependent variable		Sum of Sq.	df	Mean Sq.	F	Sig.
Count of yeast in P.D.A.	Between groups	30.802	2	15.401	9.727	0.001
	Within groups	45.917	29	1.583		
	Total	76.719	31			

Table 6. Multiple comparisons to check the significance of the differences in the yeast counts in the three regions of the gut.

Dependent variable	(I) Organ	(J) Organ	Mean difference (I-J)	S.E	Sig.
Count of yeast in P.D.A.	Gizzard	Intestine	.2500	0.5743	0.667
		Rectum	-1.9167*	0.5137	0.001
	Intestine	Gizzard	-0.25	0.5743	0.667
		Rectum	-2.1667*	0.5743	0.001
	Rectum	Gizzard	1.9167*	0.5137	0.001
		Intestine	2.1667*	0.5743	0

*The mean difference is significant at the 0.05 level.

Table 7. Mould counts along the gut of the earthworm *L. violaceus:* Total viable counts on potato dextrose agar (cfu/ml × 10^{-7}).

Replicates	Worm one				Worm two				Worm three				N	Mean	S.D
	1	2	3	4	1	2	3	4	1	2	3	4			
Gizzard	3	2	3	4	7	9	10	10	3	3	4	7	12	5.5	2.9388
Intestine	8	8	8	8	ND	ND	ND	ND	7	8	6	4	8	7.1250	1.4577
Rectum	9	8	7	9	10	7	6	7	8	7	8	7	12	7.75	1.1382

Table 8. Analysis of variance to show that the distribution of mold in the three regions of the gut is not uniform.

Dependent variable		Sum of Sq.	df	Mean Sq.	F	Sig.
Count of mold in P.D.A.	Between groups	31.875	2	15.938	3.724	.036
	Within groups	124.125	29	4.280		
	Total	156.000	31			

faecalis. And from the rectum only the following were isolated: *P. myxofasciens*, *Bacillus brevis, Klebsiella* sp, *Micrococcus varians* and *Flavobacterium aquartile.*

A total of twenty-one bacterial species were isolated from the three sample earthworms and nine fungi species (five yeast and four moulds).

Table 10. Distribution of the bacterial species in the different regions of the gut.

S/N	Bacterial species found	Organs where found			Total
	Species	Gizzard	Intestine	Rectum	
1	*Acinetobacter* sp.	1			1
2	*Alcaligans faecalis*		1		1
3	*Bacillus brevis*			2	2
4	*Bacillus ceveus*		1		1
5	*Bacillus lalerosporus*		1		1
6	*Bacillus lichenoform*		1		1
7	*Bacillus maceraus*	1			1
8	*Bacillus* sp.	1			1
9	*Corynebacterium* sp.	2	1		3
10	*Enterobacter cloacae*	1			1
11	*Erwinia salicie*		1		1
12	*Flavobacterium aquartile*			1	1
13	*Flavobacterium* sp.	1			1
14	*Klebsiella* sp.			1	1
15	*Micrococcus Inteus*		1		1
16	*Micrococcus kristinae*		1		1
17	*Micrococcus varians*			1	1
18	*Proteus myxofasciens*			2	2
19	*Proteus rennevi*	1			1
20	*Proteus vulgaris*		1		1
21	*Pseudomonas* sp.	1		2	3
	Total	9	9	9	27

DISCUSSION

The increasing trend is consistent with earlier reports (Lavelle et al., 1995).This suggests that the enteric condition of the earthworm was suitable, not only for survival of the microbes, but also for their multiplication.

From previous comparative work on counts in worm casts and parent soils, the result suggests that the enteric conditions may be more supportive of the microbes than their native soil habitat.

Even then, the distribution pattern of these microbes suggests that the diverse organisms have differential preferences for the different regions of the gut. The reasons could be due to the flushing effect of the gastric juice, availability of food nutrients, environmental and physiological factors. Microbial count is highest in the rectum. This suggests that it is the most suitable of the microhabitat. This is similar to the situation in man where, because of abundance of residual food, and non-acidic nature of rectum, microbial population is higher than in the strongly acidic stomach or strongly alkaline small intestine.

None of the yeast has the ability to digest melobiose, a disaccharide formed by an alpha linkage between galactose and glucose.

The isolation of *P. vulgaris* is of interest because under normal circumstances the bacterium harmlessly inhabits the human intestines and aid in digestion, (as it probably does in this earthworm host) but can become pathogenic and cause infections, such as urinary tract infections.

It has been estimated that the weight of leaves that fall annually in woodland varies from as little as 500 kg per ha year in alpine and arctic forests to as much as 2,500 to 3,000 kg per year in temperate forests and to as much as 5,000 to 15,000 kg per ha per year in tropical forests. Satchel (1967) calculated that if a temperate deciduous woodland has a leaf fall of 3,000 kg/ half year and if an earthworm consumes 27 mg of leaf litter per gram of earthworm, per day, which is a reasonable average expectation, then they would consume the annual leaf fall in about three months. Madge (1966) calculated that in the tropical forests in Nigeria, the litter fall was three to four times as much as in the temperate forests and suggest that earthworms were the most important animal in fragmenting and incorporating leaves and other microbiologically transformed soil and organic matter products (e.g, casts) into the soil. Although enzymes such as cellulases are exogenously and endogenously present in the gut contents of some soil invertebrates (eg, earthworm), it is claimed that they are produced by ingested microorganisms rather than by the invertebrates themselves (Urbasek, 1990).

From the view point of earthworm function in soil dynamics, as shown in the results of this study, the

diverse communities of microbial populations harboured in the gut of earthworm indicates that the mutualistic earthworm- microflora digestion system and the production of the intestinal mucus in earthworm digestion must have been greatly influenced by the presence of these microbes. The digestion of leaves litters and other organic materials which ultimately results into soil improvement and increment due to casts and cast-products incorporation is believed to be the result of the presence and the activities of these microflora in the worm's gut.

The result also indicated a systematic trend of increasing microbial biomass and a characteristic distribution pattern of microbes along the gut compartments (Gizzard, intestine and rectum). The increasing trend suggests a variation in adaptability, mechanical flushing effects of intestinal fluid and availability of food materials. The varied number of microbes in the gut shows that the rate of digestion activities also varied, since the higher the number of microbes, the faster the rate of digestion.

The general mono-locus distribution of the microbes is an indication that they prefer certain enteric microenvironments to the other. Those with multi-loci distribution could inhabit more than a microenvironment. If the microbial clusters found in the different regions are enzymatically specialized, they may be responsible for the different levels of digestion in the different regions. If so, the clusters are vital in effecting the mutualistic earthworm-microflora digestion activities.

The contribution of each of the identified species of microorganisms in earthworm digestion process is important because through their contributions earthworm facilitate the recycling soil nutrients for a greater crops yield.

Earthworm also contributes to the humification of organic materials, a function they perform along with other non-microbial organisms like mites, springtails and other arthropods. It is commonly held that while the initial processes in humification are due largely to earthworms, some of the final stages of humification are as a result of their enteric microflora because most of the evidences indicate that the process of humification are caused more by the microflora than by fauna (Feller et al., 2003).

The earthworm enteric microflora facilitates the rate at which earthworms degrade cellulose. Results from previous work (Morgan et al., 2002; Lattaud et al., 1997) established that the rate at which earthworm degrades cellulose decreases significantly when their enteric microflora are eliminated (Owa et al., 2009).

On the other hand, evidence suggests that direct *ex vivo* use of the microbes may not be as effective for cellulose decomposition as when they act *in vivo* in the earthworms (Feller et al., 2003). This is due to the fact that earthworms provide suitable environment and habitat for decomposition and transformation activities of the micro-organisms.

Considering the diversity of microbes isolated in this work (21 species of bacteria and 9 of fungi), earthworms can be said to be very effective in hosting and breeding the microbes, and likely also in inoculating the soil with these microbes.

A soil is believed to be good for planting when there are enough nutrients and humus. The process of humification increases with the population of microbes which are inoculated into the soil by the earthworms through their casting activities (Singleton et al., 2003). Therefore, the multiplication of microbes in the worm gut has an enormous economic advantage.

The enteric microbes of the earthworms are essential also because faster and better decomposition process could be carried out through the mutualistic digestive earthworm–microbe processes.

Earthworm-produced soil fertility is more economical, less labour intensive and freer of any environmental pollution than inorganic fertility. It is also preferred instead of burning the grass and farm residues. Generated smoke from burning constitutes one of the major environmental pollution and the heat from the burning process also kill soil microflora and fauna.

This study has revealed the microbial composition of the earthworm gut, the major actors in the earthworm-improved soil fertility. They are responsible for soil improvement, vegetable material transformation and incorporation. Certainly, more are to be explored concerning earthworms and their associated micro-organisms.

REFERENCES

Edwards CA, Heath GW (1963). The role of soil animals in breakdown of leaf material. In Soil Organisms. J. Doekson. van der Drift (eds). North Holland Publishing CO., Amsterdam. pp. 76-80.

Feller C, Brown G, Blanchart E, Deleporte P, Cherryanskii SS (2003). Charles Darwin, earthworms and the natural sciences: various letters from past to future. pp. 29-49

Hendriksen NB (1995). Effects of detritivoure earthworms on dispersal and survival of the bacterium *Aeromonas hydrophiula,* Acta Zoologica Fennica 196:115-118.

Lattaud C Locati S, Mora P, Rouland C (1997) Origin and activites of glucosidic enzymes in the gut of the tropical geophagus earthworms Millsonia anomala from Lamto. Pedobiologia 41:242-251.

Lavelle P, Lattaud C, Trigo D, Borois I (1995). Mutualism and biodiversity in soil. Plant Soil 30:23-33.

Loquet M, Vinceslas M (1987). Celluloyse et ligninolyse lees au tube digestif *d'Eisenia fetida* Andre Bouche. Revue d'Ecologie et Biologie du Sol. 24:558-570.

Lynch JM, Poole NJ (1979). Microbial ecology: a conceptual approach, pp. 115:127-129, Blackwell Scientific Publications, Oxford.

Madge DS (1966). How leaf litter disappears. New Scientist 32:113-115

Marks DH, Cooper EL (1977) *Aeromonas hydrophila* in the coelomic cavity of the earthworins *Lumbricus terrestris* and *Eisenia foetida*. J. Invertebr. Pathol. 29:382-383.

Morgan AJ, Winters C, Williams AF, Turner M (2002). The transformation of calcific Spherites to excretory concentrations in the earthworm calciferous glands: Did Darwin miss an ecophysiological function? In Proceedings of the Seventh International Symposium on arthworm Ecology, Cardiff, Wale September, 2002. pp. 1-6.

Owa SO, Osho BA, Dedeke GA, Aladesida AA (2009). Microbial multiplication in the gut of earthworms. J. Res. Biosci. 6(2):44-49.

Owa SO, Dedeke GA, Yeye AJ (2002). Earthworm cast characteristics under Bahama grass and the question of why earthworms cast. Afri. J. Sci. Technol. 3(1-2):33-35.

Pedersen JC, Hendrikse NB (1993). Effect of passage-through the intestinal tract of detrivore earthworms (Lumbricus spp.) on the number of selected Gram-negative and total bacteria. Biol. Fertil. Soils 16:227-232.

Satchel JE (1967). Lumbricidae. In soil Biology (eds A Surges and F. Raw) Acad. Press London and New York. pp. 259-322.

Sharpley AN, Syers JK, Springett JA (1979). Effect of surface casting earthworms on the transport of phosphorus and nitrogen in surface runoff from pasture. Soil Biol. Biochem. 11:459-461.

Singleton DR, Hendrix PF, Coleman DC, Whitman WB (2003). Identification of uncultured bacteria tightly associated with the intestine of *the earthworm Lumbricus rubellus* (Lumbricidae; Oligochaeta). Soil Biol. Biochem. 35:1547-1555.

Urbasek F (1990) Cellulose activity in the gut of some earthworms. Revue d'Ecologie et Biologie du Sol. 27(1):21-28.