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Comparative Assessment of the Aerobic and Anaerobic Microfloras of Earthworm Guts and Forest Soils

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Aerobic and anaerobic microbial potentials of guts from earthworms (*Lumbricus rubellus* Hoffmeister and *Octolasium lacteum* (Oerl.)) collected from a beech forest were evaluated. On the basis of enumeration studies, microbes capable of growth under both aerobic and anaerobic conditions were more numerous in the earthworm intestine than in the beech forest soil from which the worms were obtained. The intestine of worms displayed nearly equivalent aerobic and anaerobic microbial growth potentials; in comparison, soils displayed greater aerobic than anaerobic microbial growth potentials. Hence, the ratio of microbes capable of growth under obligately anaerobic conditions to those capable of growth under aerobic conditions was higher with the worm intestine than with the soil. Process level studies corroborated these population differentials: (i) under anaerobic conditions, worm gut homogenates consumed glucose, cellobiose, or ferulate more readily than did soil homogenates; and (ii) under aerobic conditions, worm gut homogenates consumed cellobiose or oxygen more readily than did soil homogenates. Collectively, these results reinforce the general concept that the earthworm gut is not microbiologically equivalent to soil and also suggest that the earthworm gut might constitute a microhabitat enriched in microbes capable of anaerobic growth and activity.

Earthworms are believed to play an important role in the aeration, drainage, and plant and microbial productivities of soils (11, 23). In addition, earthworms are active in litter turnover, the magnitude of which can be enormous. For example, it was calculated that *Lumbricus terrestris* consumed in a 3-month period the total annual litter fall (about 300 g m^{-2}) of a mixed forest (29). Despite such activities, the microbiology of the earthworm gut has received relatively little attention.

Although evidence for an endogenous microflora in guts of *L. terrestris* and *Octolasion cyaneum* was recently obtained by scanning electron microscopy (17), whether the microflora of the earthworm gut is different from that of soil remains a somewhat open issue, largely because of conflicting or noncomparable studies. Although early microbiological studies demonstrated that the gut of *L. terrestris* contained more culturable aerobic bacteria than did soil (1), other studies suggested that soils contained equivalent or higher numbers of culturable aerobes than did worm guts (1, 8). In a more comprehensive study, a higher number of aerobes were obtained from the guts of *L. terrestris*, *Allolobophora caliginosa*, and *Allolobophora terrestris* than from soils (27). Although an increase in the number of culturable aerobes has been obtained for consecutive gut segments (fore-, mid-, and hindgut) of *L. terrestris* and *Lumbricus rubellus*, such correlations were not obtained for *Aporrectodea caliginosa* (20, 28). *Serratia marcescens* and *Escherichia coli* were not stable to passage through *Lumbricus* species (9, 28). Although this suggests that the viability of some soil microbes might be negatively influenced by gut passage, species might reproliferate in the gut subsequent to initial inhibition or reduction in number (28). Aerobic worm gut isolates include the actinomycete *Streptomyces lipmanii* (5) and the oxalate-degrading bacterium *Pseudomonas oxalaticus* (19).

Anaerobes have not been enumerated from the worm gut. However, several anaerobic nitrogen fixers (*Clostridium butyricum*, *Clostridium beijerinckii*, and *Clostridium paraputrificum*) have been isolated from *Eisenia foetida* (4). In addition, most of the aerobic isolates from the gut of *Eisenia lucens* were identified as facultative species of *Vibrio* (25). Though a poorly explored potential, the occurrence of such anaerobic and facultative isolates suggests that the gut of the earthworm might harbor mobile anaerobic microsites in otherwise well-aerated, stationary soils. The main objective of the present study was to comparatively examine the aerobic and anaerobic potentials of the gastrointestinal microflora of earthworms derived from a beech forest near Geisberg, Germany.

MATERIALS AND METHODS

Cultivation media. Anaerobic undefined medium 1 (UM1) was modified from a previously described medium (7) and contained the following, in grams per liter (unless otherwise indicated): yeast extract, 0.5; $NaHCO_3$, 7.5; cysteine · HCl · H_2O, 0.25; $Na_2S \cdot 9H_2O$, 0.25; resazurin, 1.0 mg; mineral solution, 50.0 ml; trace element solution, 5.0 ml; B-vitamin solution, 5.0 ml. The stock mineral solution contained the following, in grams per liter: KH_2PO_4, 10.0; NaCl, 8.0; NH_4Cl, 8.0; $MgCl_2 \cdot 7H_2O$, 1.0; $CaCl_2 \cdot 2H_2O$, 0.2. The stock trace element solution contained the following, in grams per liter: sodium nitrilotriacetate, 1.5; $MnSO_4 \cdot H_2O$, 0.5; $FeSO_4 \cdot 7H_2O$, 0.1; $Co(NO_3)_2 \cdot 6H_2O$, 0.1; $ZnCl_2$, 0.1; $CuSO_4 \cdot 5H_2O$, 0.01; $AlK(SO_4)_2 \cdot 12H_2O$, 0.01; H_3BO_3, 0.01; $Na_2MoO_4 \cdot 2H_2O$, 0.01; $NiCl_2 \cdot 6H_2O$, 0.05; H_2SeO_3, 0.05; $Na_2WO_4 \cdot 2H_2O$, 0.01. The stock B-vitamin solution contained the following, in milligrams per liter: biotin, 20.0; folic acid, 20.0; pyridoxal · HCl, 20.0; lipoic acid, 50.0; riboflavin, 50.0; thiamine · HCl, 50.0; Ca–D-pantothenate, 50.0; cyanocobalamin, 50.0; *p*-aminobenzoic acid, 50.0; nicotinic acid, 50.0. The gas phase was 100% CO_2, and the pH after autoclaving approximated 6.9. Anaerobic UM1-F was UM1 supplemented with 4.5 mM ferulate.

Anaerobic undefined medium 2 (UM2) was modified from medium 10 (14) and contained the following, in grams per liter (unless otherwise indicated): glucose, 0.5; cellobiose, 0.5; soluble starch, 0.5; tryptic soy broth without dextrose, 2.0; yeast extract, 0.5; K_2HPO_4, 0.25; KH_2PO_4, 0.18; NaCl, 0.444; $(NH_4)_2SO_4$, 0.45; $CaCl_2 \cdot 2H_2O$, 0.06; $MgSO_4 \cdot 7H_2O$, 0.094; cysteine · HCl · H_2O, 0.5 (reducer); $Na_2CO_3 \cdot 10H_2O$, 10.78; resazurin (redox indicator), 1.0 mg; hemin solution, 10.0 ml (stock hemin solution contained hemin, 10.0 mg; ethanol, 50.0 ml; and 0.05 M NaOH, 50.0 ml); volatile fatty acid mixture, 3.1 ml (stock volatile fatty acid mixture solution contained acetic acid, 17.0 ml; propionic acid, 6.0 ml; butyric acid, 4.0 ml; isobutyric acid, 1.0 ml; *n*-valeric acid, 1.0 ml; isovaleric acid, 1.0 ml; D,L-α-methylbutyric acid, 1.0 ml). The gas phase was 100% CO_2. Before $NaCO_3$ was added, the pH was adjusted to 7.0 with 10 N NaOH; the pH after autoclaving approximated 6.9.

Anaerobic defined media 1-G and 1-C (DM1-G and DM1-C) were UM1 without yeast extract and contained 10 mM glucose or 10 mM cellobiose, re-

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spectively. For roll tubes, media were supplemented with 15 g of agar per liter of medium.

Aerobic UM1 and UM2 were as described above, except they did not contain bicarbonate, carbonate, cysteine, Na_2S, resazurin, and CO_2 and they were buffered with 20 mM phosphate buffer, pH 7.0. Aerobic defined medium 2-C (DM2-C) contained the following, in grams per liter (unless otherwise indicated): $Na_2HPO_4 \cdot 2H_2O$, 4.2; $NaH_2PO_4 \cdot H_2O$, 3.137; cellobiose, 1.37; mineral solution, 50 ml (see medium UM1); trace element solution, 1 ml. The trace element solution contained the following, in grams per liter: disodium EDTA dihydrate, 5.2; $FeCl_2 \cdot 4H_2O$, 1.5; $ZnCl_2$, 0.07; $MnCl_2 \cdot 4H_2O$, 0.1; H_3BO_3, 0.062; $CoCl_2 \cdot 6H_2O$, 0.19; $CuCl_2 \cdot 2H_2O$, 0.017; $NiCl_2 \cdot 6H_2O$, 0.024; $NaMoO_4 \cdot 2H_2O$, 0.036. The pH of DM2-C after autoclaving approximated 6.8. For agar plates, media were supplemented with 15 g of agar per liter of medium. The gas phase of all aerobic cultures was air.

Homogenization buffers. The aerobic homogenization buffer contained the following, per liter: mineral solution, 50 ml (see medium UM1); $Na_2HPO_4 \cdot 2H_2O$, 1.678 g; $NaH_2PO_4 \cdot H_2O$, 0.948 g. The anaerobic homogenization buffer was prepared anaerobically (16) and contained the following, per liter: mineral solution, 50 ml (see medium UM1); $Na_2HPO_4 \cdot 2H_2O$, 1.678 g; $NaH_2PO_4 \cdot H_2O$, 0.948 g; resazurin, 1.0 mg; cysteine $\cdot$ HCl $\cdot H_2O$, 0.25 g; $Na_2S \cdot 9H_2O$, 0.25 g. The gas phase of the anaerobic homogenization buffer was 100% N_2. The pHs of both buffers after autoclaving approximated 6.9.

Collection of earthworms and soils. The sampling site was a beech forest near Geisberg, Germany. The soil is classified as a chromic luvisol, and the parent material is limestone (32). Soil was collected from the A_h horizon (0 to 5 cm), which was a silty loam with a pH of 6.0 to 6.5 (determined with 0.01 M $CaCl_2$). Most earthworms were collected from the A_h horizon or under the litter layer; some were also obtained from the A_l horizon (5 to 13 cm). Adult earthworms and soil were collected in separate sterile vessels, returned to the laboratory, and stored overnight at 5°C. Earthworms were transported and stored in soil. Identification of earthworms was according to published protocols (3, 31).

Preparation of worm gut and soil homogenates. Homogenates were prepared by using aseptic techniques. Worms were washed with sterile (autoclaved) tap water, weighed, and subsequently narcotized with 100% CO_2. The surface was sterilized by brief flaming with ethanol; on the basis of plate counts, this protocol destroyed essentially the entire culturable surface microflora. Unless otherwise indicated, all subsequent procedures were performed in a Mecaplex anaerobic chamber (Grenchen, Switzerland) with a 100% N_2 gas phase. The gut section posterior of the gizzard was dissected out, weighed (150-mg average wet weight), and homogenized (for 5 min with a Vortex mixer) in homogenization buffer (5 or 10 ml) containing glass beads (3 mm). For preparation of gut content homogenates, the gut content was gently pressed out through the anus with sterile forceps; homogenization was as described for gut sections. Anaerobic soil homogenates were prepared in the same way as gut homogenates with the exception that soil was not processed in the anaerobic chamber but was degassed with 100% N_2 before the addition of anaerobic homogenization buffer. For O_2 consumption studies, gut and soil homogenates were prepared with aerobic homogenization buffer on the laboratory bench.

Enumeration of gut and soil bacteria. Anaerobic roll tubes (1.8 by 15 cm) were used for the enumeration of microbes capable of anaerobic growth and were prepared by modified Hungate technique (16). Aerobic agar plates (diameter, 8.5 cm) were used for the enumeration of microbes capable of aerobic growth. For CFU, anaerobic gut and soil homogenates were serially diluted (1:10) in anaerobic homogenization buffer; these dilutions were utilized for inoculation of anaerobic roll tubes. Because it was not technically possible to prepare both aerobic and anaerobic homogenates from the gut of a single individual (i.e., the amount of gut material was limiting), the anaerobic dilutions were utilized for inoculation of aerobic agar plates. On the basis of trials with soil homogenates, this procedure did not influence the aerobic enumeration results. Agar plates and roll tubes were incubated at 20 or 30°C, as indicated. Colonies on each plate or in each roll tube were counted daily until colony counts reached a stable plateau. Each CFU value is the average from triplicate plates or roll tubes. Hypha-forming, funguslike colonies were not enumerated; viable counts thus reflect only bacterium- and yeastlike colonies.

Substrate utilization and process studies. Gut sections and gut contents contained higher amounts of organic carbon than soils (Table 1). To compensate for the fact that it was not possible to determine the amount of organic carbon of freshly prepared homogenates prior to inoculation (because of the time and quantity of gut material required), the dry-weight amounts of gut sections and gut contents used in the substrate utilization and process studies were less than that of the soil.

For assessment of the anaerobic utilization of cellobiose, glucose, or ferulate, serum bottles (60 ml) containing DM1-C (21 ml), DM1-G (24 ml), or UM1-F (24 ml) were inoculated with homogenates (1 or 2 ml). For assessment of the aerobic utilization of cellobiose, Erlenmeyer flasks (100 ml) containing 24 ml of DM2-C were inoculated with 2 ml of homogenates and shaken on a rotary shaker (100 rpm). For O_2 consumption studies, 2 ml of homogenates was transferred to sterile, rubber-stoppered, screw-cap culture tubes (16 ml); tubes were incubated horizontally without shaking. The temperature of incubation in all cases was 20°C.

Analytical methods. Glucose, cellobiose, and soluble products were quantitated with a Hewlett-Packard 1090 series II high-performance liquid chromatograph (HPLC) equipped with an HP 1047A refractive index detector, an HP 3396 series II integrator (Hewlett-Packard, Palo Alto, Calif.), and an Animex Ion Exclusion HPX-87H column (300 by 7.8 mm; Bio-Rad, Richmond, Calif.). The column temperature was 60°C; the mobile phase was 0.01 N H_2SO_4 at a flow rate of 0.8 ml/min. Ferulate was quantitated by HPLC as described above, except a fermentation monitoring column (150 by 7.8 mm; Bio-Rad) and an HP series 1050 UV detector (210 nm) were used for separation and detection. Culture samples were clarified by microcentrifugation and microfiltration prior to chromatographic analysis. Hydrogen (H_2) and O_2 were quantitated with a Hewlett-Packard 5980 series II gas chromatograph equipped with a molecular sieve (Alltech, Unterhaching, Germany) column (length, 2 m; inner diameter, 3.2 mm) and a thermal conductivity detector with argon as the carrier gas (flow rate, 33 ml/min); the injector temperature was 150°C, the column temperature was 60°C, and the detector temperature was 175°C. Before gas samples were taken with a sterile argon-flushed syringe, tubes or bottles were shaken for 30 s to ensure equilibration between the liquid and gas phases. The total gas pressures of tubes or bottles were measured with a Ballmoos (Horgen, Switzerland) DMG 2120 needle manometer. Total carbon and nitrogen were quantitated with an element analyzer (CHN-O-rapid; Foss-Heraeus, Hanau, Germany), and organic carbon was calculated as the difference between total carbon and inorganic carbon after the organic carbon was eliminated by high temperature (500°C, 10 h). Dry weights of soils and guts were obtained by weighing before and after drying at 105°C for 16 h. Percent moisture content was calculated as the percent weight loss from drying.

TABLE 1. Carbon, nitrogen, and moisture contents of soil, earthworm gut sections, and earthworm gut contents[a]

Material	C and N contents (g/kg [dry wt])			C/N ratio[a]	% Moisture (no. of samples)
	$C_{organic}$	$C_{inorganic}$	N_{total}		
Soil ($n = 2$)[b]	75	0	6	12.5	36 (52)
L. rubellus gut sections ($n = 13$)[c]	304	3	39	7.9	71 (25)
L. rubellus gut contents ($n = 35$)[c]	256	3	30	8.7	60 (4)
O. lacteum gut sections ($n = 11$)[c]	224	1	31	7.3	70 (9)
O. lacteum gut contents ($n = 12$)[c]	128	0	13	10.2	57 (3)

[a] The C/N ratio was calculated as C_{total}/N_{total}, where $C_{total} = C_{organic} + C_{inorganic}$.

[b] Value is the average of replicate analyses, where n is the number of samples analyzed.

[c] n is the number of gut sections or gut contents pooled for analysis.

RESULTS

Population differentials of culturable microfloras. On a dry-weight basis, the earthworm gut yielded higher numbers of culturable microbes than did soil (Table 2). In addition, earthworm gut sections had a significantly higher number of culturable anaerobes than soil, this pattern being somewhat less pronounced for organisms capable of aerobic growth. The differential between the anaerobic viable counts from gut sections and soils was independent of the enumeration medium used (i.e., undefined versus defined media).

Gut homogenate-derived colonies developed somewhat faster than did those derived from soil homogenates (data not shown). No hypha-forming colonies were observed in anaerobic roll tubes; efforts to isolate anaerobic fungi from the earthworm gut were not successful. Furthermore, the number of hypha-forming colonies on aerobic agar plates was very low for both soil and gut (approximately 100-fold less than the colonies enumerated). Use of a modified Sweet-E broth (15) or a modified complete carbohydrate medium (24) or enrichment of media with ferulate or vanillate did not significantly increase viable counts or alter enumeration patterns; in addition, anaerobic roll tubes consistently yielded higher viable counts than

TABLE 2. CFU obtained from earthworm gut sections and soils[a]

Sampling	Source of inoculum	CFU/g (dry wt) (10^5)[b]	
		Anaerobic	Aerobic
A	*L. rubellus* gut section ($n = 2$)	58,950 (4,840)	15,150 (3,060)
	O. lacteum gut section ($n = 1$)	3,820 (11)	2,160 (142)
	Soil ($n = 1$)	13 (3)	89 (131)
B	Soil ($n = 1$)	6 (6)	105 (65)
C	*L. rubellus* gut section ($n = 2$)	202	241
	O. lacteum gut section ($n = 1$)	44	180
	Soil ($n = 1$)	3	125
D	Soil ($n = 1$)	6	287
E	*L. rubellus* gut section ($n = 2$)	1,370	ND[c]
	Soil ($n = 2$)	1	ND

[a] Media used were as follows: samplings A, C, and D, UM1; sampling B, UM2; sampling E, DM1-G. In cases where $n = 2$, the value is the average of both samples. Sampling times were as follows: A, 24 August 1992; B, 7 September 1992; C, 26 October 1992; D, 10 November 1992; E, 12 October 1993. The temperature of incubation was 20°C. The average relative standard deviation of the triplicate determinations was ± 12.8%.

[b] CFU values in parentheses were obtained at an incubation temperature of 30°C.

[c] ND, not determined.

did the most-probable-number technique (with anaerobic liquid culture media) (data not shown).

Comparative evaluation of the overall enumeration results indicated that microbes capable of growth under anaerobic conditions were approximately 2 to 3 orders of magnitude more numerous in the earthworm intestine than in the soil (Table 3). Soils had greater aerobic than anaerobic potentials, while the intestines of worms harbored nearly equivalent numbers of microbes culturable under either anaerobic or aerobic conditions. Thus, the ratio of microbes capable of growth under obligately anaerobic conditions to those capable of growth under aerobic conditions was higher in the worm intestine than in the soil. This correlation between worm gut and soil microfloras is reflected in the comparative ratio in the last column of Table 3. Although this comparative ratio was highly variable, it exceeded a value of 10 for each worm examined. A value of less than 1 would indicate a comparative predominance of microbes capable of aerobic growth in the gut relative to the soil.

Diverse microflora of gut. Electron microscopy of earthworm guts reveals a complex, diverse microflora (17). By light microscopy, the gut homogenates in the present study were also rich in diverse morphological types, and enrichments contained mixtures of rods, cocci, and spore-forming microbes. A single dominant morphological type was not apparent.

TABLE 3. Comparative analysis of viable counts of microorganisms obtained from earthworm gut sections and soils[a]

Source of inoculum	Avg CFU/g (dry wt) (10^5)		Ratio of A/B (C)	Ratio of C_{gut}/C_{soil}
	Anaerobic (A)	Aerobic (B)		
L. rubellus gut section	20,174 ($n = 6$)	7,696 ($n = 4$)	2.62	79 (83)[b]
O. lacteum gut section	1,932 ($n = 2$)	1,170 ($n = 2$)	1.65	50 (36)[b]
Soil	5 ($n = 6$)	152 ($n = 4$)	0.03	NA[c]

[a] Values are based on data derived from Table 2 (data obtained at an incubation temperature of 30°C were not used).

[b] Value in parentheses is that obtained when sampling E was factored out.

[c] NA, not applicable.

Anaerobic utilization of model substrates by gut and soil microfloras. Soil and gut homogenates were examined for their comparative potentials to consume the model substrates glucose, cellobiose, and ferulate. Low-molecular-weight substrates such as monomeric carbohydrates have been observed in worm guts (26). In addition, by HPLC, trace levels of glucose and cellobiose were detected in gut homogenates.

The onset of the anaerobic utilization of cellobiose and glucose was consistently more rapid with earthworm gut section homogenates than with soil homogenates (Fig. 1A and B, respectively). When DM1-G was inoculated with the same number of soil- or gut section-derived glucose utilizers (determined by both CFU analysis and the most-probable-number technique), the onset of anaerobic glucose utilization was similar for both soil and gut section homogenates (data not shown). This result indicated that the higher number of culturable anaerobes observed in the earthworm gut might be directly correlated, on a dry-weight basis, with the increased initial capacities of gut homogenates to consume certain substrates.

Glucose- and cellobiose-derived products by gut section homogenates were lactate, ethanol, formate, acetate, butyrate, succinate, and hydrogen (Fig. 2). Although the initial kinetics of substrate utilization differed between gut and soil homogenates, both gut and soil homogenates yielded similar product patterns from glucose and cellobiose (data not shown). In contrast to glucose and cellobiose, ferulate, a model lignin derivative, was consumed at a significantly lower rate by gut section and soil homogenates (Fig. 1C). Although ferulate utilization was initially more rapid with gut section homogenates, ferulate was not totally consumed by either soil or gut section homogenates, indicating a nutritional limitation or inhibition relative to the turnover of ferulate. The products formed during the anaerobic utilization of ferulate were complex. Identified soluble products were hydroferulate, acetate, and formate. Not detected were caffeate, hydrocaffeate, 2-methoxy-4-vinylphenol and 4-ethyl-2-methoxyphenol, catechol, guaiacol, and vanillate. However, on the basis of absorption intensities, a nonidentified aromatic compound was observed by HPLC.

Aerobic activities of gut and soil microfloras. Under aerobic conditions, cellobiose was also utilized more readily by gut section homogenates than by soil homogenates (Fig. 3A). In addition, homogenates from gut sections or gut contents of *L. rubellus* displayed an immediate potential to consume O_2; in contrast, soil homogenates consumed relatively little O_2 in the absence of supplemental energy or carbon sources over the assay period (Fig. 3B and C). That gut sections and gut contents displayed similar activities relative to O_2 uptake suggests that the potential to do so was not dependent on the organic matter derived from the intestinal wall.

DISCUSSION

The collective results suggest that the earthworm gut and soil of the beech forest studied were not equivalent relative to culturable microflora. The microbial community of the earthworm gut was more responsive than that of the soil, reinforcing the concept that the earthworm gut might be a specialized microhabitat of enhanced microbial activities in forest soils. The dissimilar physicochemical properties of the earthworm

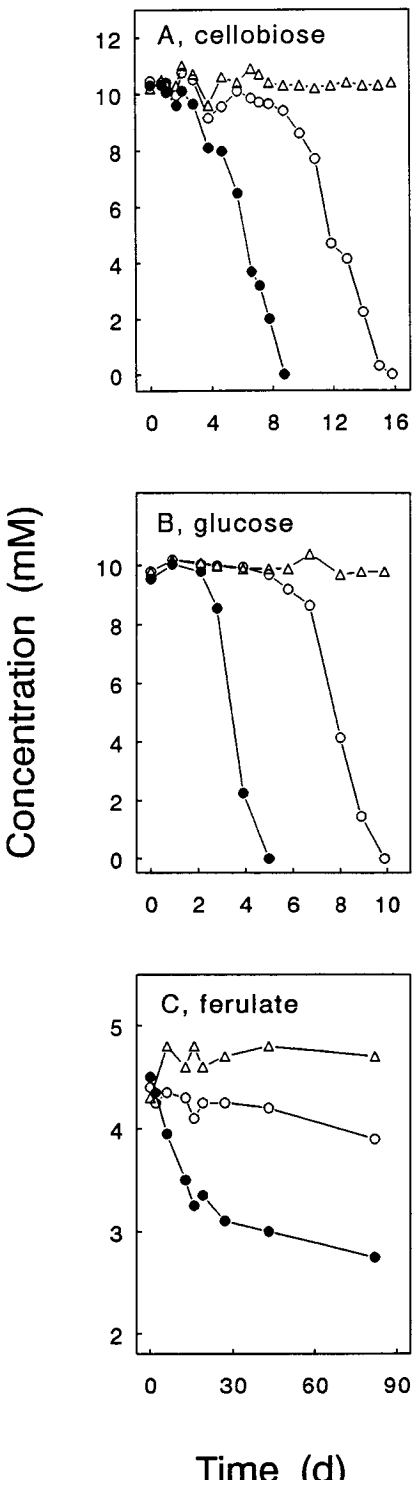

FIG. 1. Anaerobic utilization of cellobiose, glucose, and ferulate by soil homogenates (○), *L. rubellus* gut section homogenates (●), and uninoculated controls (△). Inocula were as follows (milligrams of homogenate dry weight per milliliter of reaction mixture): (A) 1.27 (gut section; similar results were obtained with gut sections from *O. lacteum*), 3.75 (soil); (B) 0.94 (gut section; similar results were obtained in replicate experiments), 2.2 (soil); (C) 0.29 (gut section; similar results were obtained in replicate experiments), 0.55 (soil). Sampling times were 11 January 1994 (A), 23 June 1993 (B), and 29 March 1993 (C). Values are the averages from duplicate serum bottles inoculated with the same homogenate; control values are from a serum bottle inoculated with sterile homogenate buffer.

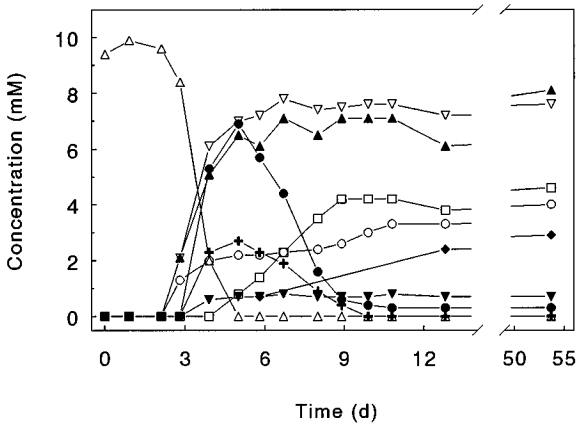

FIG. 2. Products formed from glucose under anaerobic conditions by a gut section homogenate from *L. rubellus*. Symbols: △, glucose; ●, lactate; ▲, ethanol; ▽, formate; ○, acetate; □, butyrate; ▼, succinate; +, pyruvate (note: pyruvate and oxaloaceate could not be distinguished by HPLC); ◆, hydrogen (measured only during the last incubation period). The inoculum was 0.94 mg (dry weight) of gut section homogenate per ml of reaction mixture. Glucose was not consumed in an uninoculated control. Similar product patterns were observed in both replicate experiments and when cellobiose was used instead of glucose. The sampling time was 23 June 1993.

gut and soil might be correlated to the differentials between soils and guts. The intestine contained about twice as much water as did the soil (Table 1), a factor that might be the most rate-limiting factor relative to the activity of soil- and earthworm-associated microbes (10, 28, 32). In addition, the increased organic carbon and nitrogen content of the worm gut (Table 1) may also stimulate microbial activity. However, in both the enumeration and process studies, essentially only quantitative or kinetic differences were observed between the culturable microfloras of soils and earthworm guts. It is therefore not possible to conclude that the differences observed were related to endogenous microbes that selectively colonize the gut.

The guts of the earthworms examined contained similar numbers of culturable anaerobes and aerobes, while soils appeared to have a higher number of microbes capable of aerobic growth. Although earthworms can feed on and thereby kill microorganisms during gut passage (9, 13), it is doubtful that feeding or killing could consistently select for either aerobic or anaerobic microorganisms. It is therefore easy to speculate that certain ingested microbes find better environmental conditions in the intestine relative to that of the soil and hence proliferate during gut passage. However, the extent to which microbes might proliferate during gut passage is still an open issue since the extractability and culturability of soil and gut microfloras may not be equivalent. Since some of the anaerobic population differentials between gut contents and soils would require a substantial number of doublings (approximately 10) to achieve, gut passage may selectively activate or prime a portion of the soil microflora relative to culturability. In this regard, the amount of microbial biomass in soils and 9-h-old earthworm casts may not differ substantially (6). Molecular probes may be of special use relative to further resolving differences between the microfloras of soils and the intestinal tract of the earthworm.

The data from samplings A and B (Table 2) suggest that the microbial growth potentials of the worm gut were more sensitive to elevated mesophilic temperatures than were those of soil. Forest soils from this region experience relatively mild temperatures (approximately 15°C in midsummer). Although

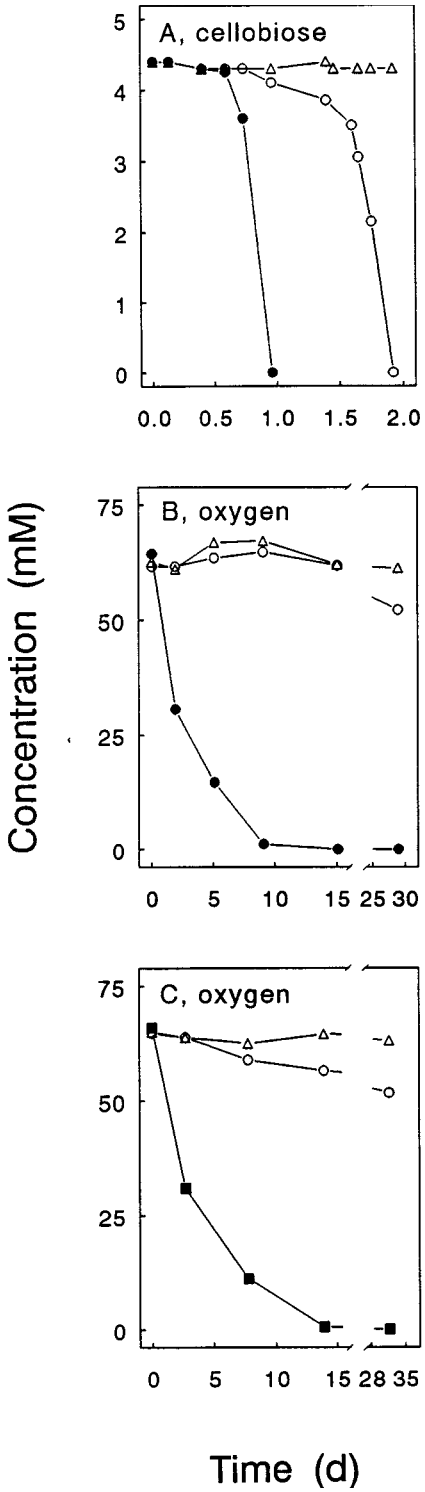

FIG. 3. Aerobic utilization of cellobiose and consumption of oxygen without supplemented carbon or energy source by soil homogenates (○), *L. rubellus* gut section homogenates (● [A and B]), *L. rubellus* gut content homogenates (■ [C]), and uninoculated controls (△). Inocula were as follows (milligrams of homogenate dry weight per milliliter of reaction mixture): (A) 0.64 (gut section; similar results were obtained with gut sections from *O. lacteum*), 1.34 (soil); (B) 4.2 (gut section; similar results were obtained in replicate experiments), 14.9 (soil); (C) 8.9 (gut contents; similar results were obtained in replicate experiments), 16.5 (soil). Sampling times were 25 May 1994 (A), 23 February 1993 (B), and 29 March 1993 (C). Values are the averages from duplicate serum bottles or culture tubes inoculated with the same homogenate; control values are from either a serum bottle or a culture tube inoculated with sterile homogenization buffer.

further studies would be required to evaluate the differential effects of temperatures on soil- and gut-associated microbial activities, the present results illustrate that comparative enumeration studies should take into consideration the potential impact of temperatures in excess of those typical of the region under study.

More culturable microbes were observed in the gut of *L. rubellus* than in that of *Octolasium lacteum*. Although more data are needed to substantiate this observation, it is interesting to note that food passage through the gut is slower in *L. rubellus* than in *O. lacteum* (12, 23). In addition, microbes should theoretically find more enriched growth conditions in the intestine of *L. rubellus* compared with *O. lacteum* since *L. rubellus* is epigeic and feeds more on organic matter (litter) above the mineral layers of soils; *O. lacteum* is endogeic and feeds in mineral soils which contain a lower organic carbon content (12, 18, 23). The increased organic carbon content of the gut of *L. rubellus* relative to that of *O. lacteum* corroborates this possibility (Table 1).

The higher potential of worm guts to rapidly consume oxygen suggests that the organic carbon of the worm gut might be qualitatively different, i.e., more readily utilizable, than that of the soil. Such differences in organic carbon may be due to (i) selective feeding of the worms and/or (ii) secretion of mucus into the gastrointestinal tract (22, 30). It is likely that worms ingest oxygen together with food particles and that the oxygen concentration decreases from the anterior gut to the posterior gut due to microbial respiration during passage through the gut. A second oxygen gradient might also occur from the gut wall (blood vessels) to inner gut sites. These considerations might be correlated to the increased culturable aerobic (as well as anaerobic) population of the earthworm gut observed in the present study, as well to the earlier observation that hindguts from *L. terrestris* harbored a 100-fold-higher number of culturable aerobes than did foreguts (27).

It has recently been speculated that the classic anaerobic microhabitat of the termite gut may be characterized by fluctuating differences between oxic and anoxic microzones and activities (2, 21). Despite the increased aerobic growth potentials of the earthworm gut microflora, the number of organisms capable of anaerobic growth was more significantly elevated in worm guts relative to that of the soil (Table 3). The glucose-derived product profile obtained under anaerobic conditions (Fig. 2) mimicked a complex acid-solvent fermentation profile. In many anaerobic habitats, H_2 is believed to form important trophic links in the anaerobic community between acetogenic, methanogenic, and sulfate-reducing processes (34). Since H_2 was a stable end product from glucose (Fig. 2), the potential of the worm gut microflora to consume H_2 under anaerobic conditions appeared to be minimal. Consistent with this observation, H_2 was also not utilized by H_2-supplemented, anaerobically incubated gut section homogenates (data not shown). Further study would be required to resolve habitat (type of soil) and seasonal variabilities of the earthworm gut microflora.

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