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PROJECT 1

Utilization of Methoxy Compounds by Homoacetogen Enrichments

PROJECT 2

Chemotaxis Studies of Termite Gut Microbes

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PROJECT 1: Utilization of Methoxy Compounds by Homoacetogen Enrichments

Abstract

Some homoacetogens have the ability to utilize the methoxy groups of methoxy compounds to produce acetate. It was hypothesized that the homoacetogen enrichments from School Street Marsh may be capable of utilizing methyl-tert-butyl ether (MTBE) and other methoxy compounds. Liquid enrichments of homoacetogen subcultures obtained from the class were amended with 2mM of MTBE, 2-methoxypropene, 3-methoxycyclohexanecarboxylic acid, 1,1,2-trimethoxyethane, and trimethoxybenzoate. Because the cultures were assumed to also have methanogens, methane was measured as a possible end-product of substrate transformation. The results of the experiment showed that 4 of the 6 enrichments were capable of utilizing 2-methoxypropene and trimethoxybenzoate based on methane production relative to the negative controls. It was concluded that acetone may be an intermediate product that links the processes of homoacetogenesis and methanogenesis. However, a crude mass balance indicates that there is either untransformed substrate left in culture or intermediate products. The lack of methane production in the other treatments was not interpreted as inability to use the substrates because acetate was not measured. It is possible that homoacetogens in the other treatments could produce acetate, but methanogens may be inhibited by the substrates and incapable of transforming the acetate to methane.

Introduction

Acetogens are gram-positive, strict anaerobic members of the Bacteria They synthesize acetate from two C_1 units (Biology of the Prokaryotes, 1999):

$2CO_2 + 4H_2 \dashrightarrow acetate^- + 2H_2O + H^+$ $\quad \Delta G^{\circ\prime} = -95$ kJ/mol acetate

4 Methanol $+ 2CO_2 \dashrightarrow 3\ acetate^- + + 3H^+ + 2H_2O$ $\quad \Delta G^{\circ\prime} = -70$ kJ/mol acetate

$4CO + 2H_2O \dashrightarrow acetate^- + 2CO_2 + H^+$ $\quad \Delta G^{\circ\prime} = -175$ kJ/mol acetate

Some acetogens can activate molecules than contain more than more carbon and hydrolyze or oxidize them to precursors to acetogenesis. An example is *Clostridium aceticum* which can degrade glucose (Biology of the Prokaryotes, 1999). Homoacetogens are a special class of acetogens that use carbon monoxide dehydrogenase in the reductive direction. Some can participate in sugar fermentation and in the degradation of special substrates such N-methyl compounds or methoxylated phenols (Biology of the Prokaryotes, 1999). In methoxy compounds, the key reaction is the cleavage of the ether bond, followed by transfer of the methyl group to tetrahydrofolate. This reaction is catalyzed by the enzyme *O*-demethylase. Methyl tetrahydrofolate is: 1) oxidized to CO_2, providing reducing equivalents for the reduction of CO_2 to CO, AND 2) combined with CO and CoA to acetyl-CoA, which is converted to acetate (Kaufmann et al., 1997). The ability to cleave the ether bond in methoxy compounds might suggest that homoacetogens can activate methyl tert-butyl alcohol (MTBE).

MTBE is a gasoline oxygenate that is extremely water soluble and moderately volatile in the environment. MTBE contamination of surface waters, groundwater soils and sediments has

raised concern for public health. The U.S. Environmental Protection Agency classifies MTBE as a possible carcinogen (Hanson et al., 1999). Some studies have shown biodegradation of MTBE by mixed consortia or pure cultures of bacteria (Salanitro et al., 1994; Steffan et al., 1997; Mo et al., 1997; Hanson et al., 1999). However, the ability of homoacetogens to utilize MTBE has not been studied. Because MTBE contamination has been attributed in many cases to leaking underground storage tanks, the possibility of transformation of MTBE by anaerobic bacteria might be of interest given that contaminated groundwater systems are often anaerobic. This study addressed the potential for homoacetogen enrichments from School Street Marsh to degrade MTBE and other methoxy compounds. The inclusion of other compounds in the study was to provide the opportunity to examine compounds that differed in steric hindrance near the methoxy group. In addition to the methoxy compound study, the ability of methanogenic cultures to utilize tert-butyl-alcohol vs. n-butanol was also addressed.

Methods

Preparation of the Homoacetogenic Enrichments

The inocula for the methoxy compound study were obtained from subcultures of homoacetogen enrichments growing on trimethoxybenzoate. The original homoacetogen enrichments were samples from School Street Marsh. Subcultures were chosen based on acetate production and by the morphological diversity of the culture. The enrichments chosen had high acetate values (>10 mM) and the greatest diversity of morphotypes. Enrichments were grown in liquid freshwater media that was prepared in a Widdel flask (See Appendix). Nine mL of the media was dispensed under N_2/CO_2 (80/20%) into balch tubes and sealed with rubber stoppers. Each set of enrichments had 7 treatments (See Table 1). The culture tubes were inoculated with one mL of June 24, 2000 subcultures. Substrates were added (2 mM final concentration), and the culture tubes were gently inverted to mix the culture (See Table 1). Time-zero samples were taken by removing 0.92 mL and adding 0.1 mL of 3 mM perchloric acid in an eppendorf tube (wanted at least 1 mL of supernatant for HPLC analysis). The samples were centrifuged at 14000 rpm for 10 minutes. The supernatant was removed and frozen for later analysis of acetate. Culture tubes were incubated at room temperature and then placed in the 30 °C incubator in the dark to enhance growth conditions.

Table 1

June 24, 2000 Subcultures Used for the New Enrichments	Substrate
1. Amy Callaghan	Methyl-tert-Butyl Ether
2. Jamie McCance	Para-methoxybenzoic acid
3. Annette Zaar	2-methoxypropene
4. Kristin Lester	1,1,2-trimethoxyethane
5. Dawn Ward	Trimethoxybenzoic acid (+ Control)
6. Fresh sample from School Street	Negative Control – No substrate

The time zero samples were defrosted and analyzed for acetate by HPLC (Waters 2690 Alliance, Waters 410 Refractive Index Detector, Shodex KC811 column; isocratic; flow = 0.8 mL/min; column temperature = 50 °C; detector temperature = 30 °C; 3 mM perchloric acid eluent). After 7 days, enrichment 5 growing on 2-methboxypropene was sub-cultured and amended with 5 mM 2-methoxypropene. Time-zero methane samples were taken for the new cultures and analyzed by GC-FID. (Varian 3800, Chrompack Poraplot U column, 40 °C isocratic, injector temperature = 200 °C, column flow = 16.8 mL/min, detector temp = 300 °C). After 12 days, all of the enrichments were sampled for methane.

Microscopy

Wet mounts were made of cultures that appeared to be growing on 2-methoxypropene and trimethoxybenzoate. Images were taken with a Zeiss Axioplan 2 microscope. Additionally, other cultures were checked for growth by looking at wet mounts.

Preparation of the Methanogenic cultures

The methanogenic enrichments were prepared with the same freshwater media recipe as the homoacetogen enrichments (See Appendix). Nine mL of media was dispensed under N_2/CO_2 gas into balch tubes and sealed with stoppers with aluminum crimp tops. The three inocula were: 1) untreated septage from the Falmouth Water Sewage Treatment Plant, 2) School Street Marsh, 3) and tree soil. Enrichments were amended with either 2 mM tert-butyl alcohol, 2 mM n-butanol (positive control) or no substrate. Tubes were gently inverted to distribute the substrate. Time-zero methane samples (0.5 mL headspace) were taken and analyzed by GC-FID (Varian 3800, Chrompack Poraplot U column, 40 °C isocratic, injector temperature = 200 °C, column flow = 16.8 mL/min, detector temperature = 300 °C). Cultures were stored at room temperature until they were placed in the 30 °C incubator. Methane samples were taken 6 days and 12 days after the experiment started.

Results

Homoacetogenic Enrichments

After two days there was visible growth in all of the culture tubes amended with trimethoxybenzoate (the positive control). The culture tubes appeared cloudy. However, enrichment 3 did not appear to have as much growth as the other enrichments. By day 6, there appeared to be growth in enrichments 5 and 1 growing on 2-methoxypropene. It was unclear whether the cloudiness was due to growth or a precipitate of the 2-methoxypropene. Methane was detected in the 2-methoxypropene tube of enrichment 5, and we concluded that the cloudiness was due to growth. Enrichment 5 was sub-cultured into fresh media amended with 5 mM 2-methoxypropene. Methane was measured two days later and was 4.37 and 4.86 % for the duplicate subcultures. The results of the final methane analyses are shown in Table 3. Enrichments 1, 2, 3, and 5 utilized both trimethoxybenzoate and 2-methoxypropene. Enrichments 4 and 6 failed to convert any substrates to methane.

Table 2. Results of the Methoxy Compound Study

Enrichment	Substrate Treatment	% CH_4 (after 12 Days)
1a	MTBE	0.34
1b	p-methoxybenzoate	0.31
1c	3-methoxycyclohexanecarboxylic acid	0.32
1d	2-methoxypropene	1.46
1e	1,1,2-trimethoxyethane	0.35
1f	Trimethoxybenzoate (+ control)	2.24
1g	No substrate (- control)	0.33
2a	MTBE	0.01
2b	p-methoxybenzoate	0.03
2c	3-methoxycyclohexanecarboxylic acid	0.02
2d	2-methoxypropene	1.39
2e	1,1,2-trimethoxyethane	0.04
2f	Trimethoxybenzoate (+ control)	0.56
2g	No substrate (- control)	0.02
3a	MTBE	0.02
3b	p-methoxybenzoate	0.04
3c	3-methoxycyclohexanecarboxylic acid	0.02
3d	2-methoxypropene	1.48
3e	1,1,2-trimethoxyethane	0.10
3f	Trimethoxybenzoate (+ control)	0.11
3g	No substrate (- control)	0.02
4a	MTBE	0
4b	p-methoxybenzoate	0.02
4c	3-methoxycyclohexanecarboxylic acid	0.01
4d	2-methoxypropene	0
4e	1,1,2-trimethoxyethane	0
4f	Trimethoxybenzoate (+ control)	0.01
4g	No substrate (- control)	0
5a	MTBE	0.04
5b	p-methoxybenzoate	0.05
5c	3-methoxycyclohexanecarboxylic acid	0.05
5d	2-methoxypropene	3.87
5e	1,1,2-trimethoxyethane	0.04
5f	Trimethoxybenzoate (+ control)	0.44
5g	No substrate (- control)	0.03
5aa-transfer	2-methoxypropene	4.37
5bb-transfer	2-methoxypropene	4.86
5cc-no inoculum	2-methoxypropene	0
5dd-no inoculum	2-methoxypropene	0
6a	MTBE	0
6b	p-methoxybenzoate	0
6c	3-methoxycyclohexanecarboxylic acid	0
6d	2-methoxypropene	0
6e	1,1,2-trimethoxyethane	0
6f	Trimethoxybenzoate (+ control)	0.01
6g	No substrate (- control)	0.01

Microscopy

Enrichment 5 amended with 2-methoxypropene appeared to have 2 types of *Methanosarcina*-like cells (Figure 1). The smaller type clustered together more than the larger morphotype. Some of the larger cells seemed to have cell division planes that were non-

perpendicular (Figure 2). Both of the *Methanosarcina*-like cells had F-420 and F-350. In addition to these morphotypes, spirochetes, motile rods (single and diplo) and several other bacteria were observed. Enrichment 5 amended with trimethoxybenzoate (TMB) also had the larger *Methanosarcina*-type archaea (Figure 3). Protozoa were also observed (Figure 3). Although the protozoa were documented only for enrichment 5, they were observed in other enrichments. It was unclear whether single cells of the smaller type of *Methanosarcina* were present. No clusters of the smaller archaea were observed. However, unlike the 2-methoxypropene enrichment, the TMB enrichment had clusters of bacteria with several morphotypes (Figure 4).

Enrichment 1 amended with TMB had *Methanosarcina*-like cells that appeared to be like the larger type in a different growth phase because they were smaller (Figure 5). Division planes were not visible. The consortia also consisted of clusters of bacteria of several morphotypes including fat rods, tapered rods, single and diplo rods, and possibly spirilla (Figure 6).

Enrichment 1 amended with 2-methoxypropene had cells that looked like the smaller type of *Methanosarcina* that form clusters (Figure 7). This enrichment also had clusters of other bacteria of several morphotypes (Figure 8).

Figure 1: Two types of *Methanosarcina*-type cells in Enrichment 5 amended with 2-methoxypropene.

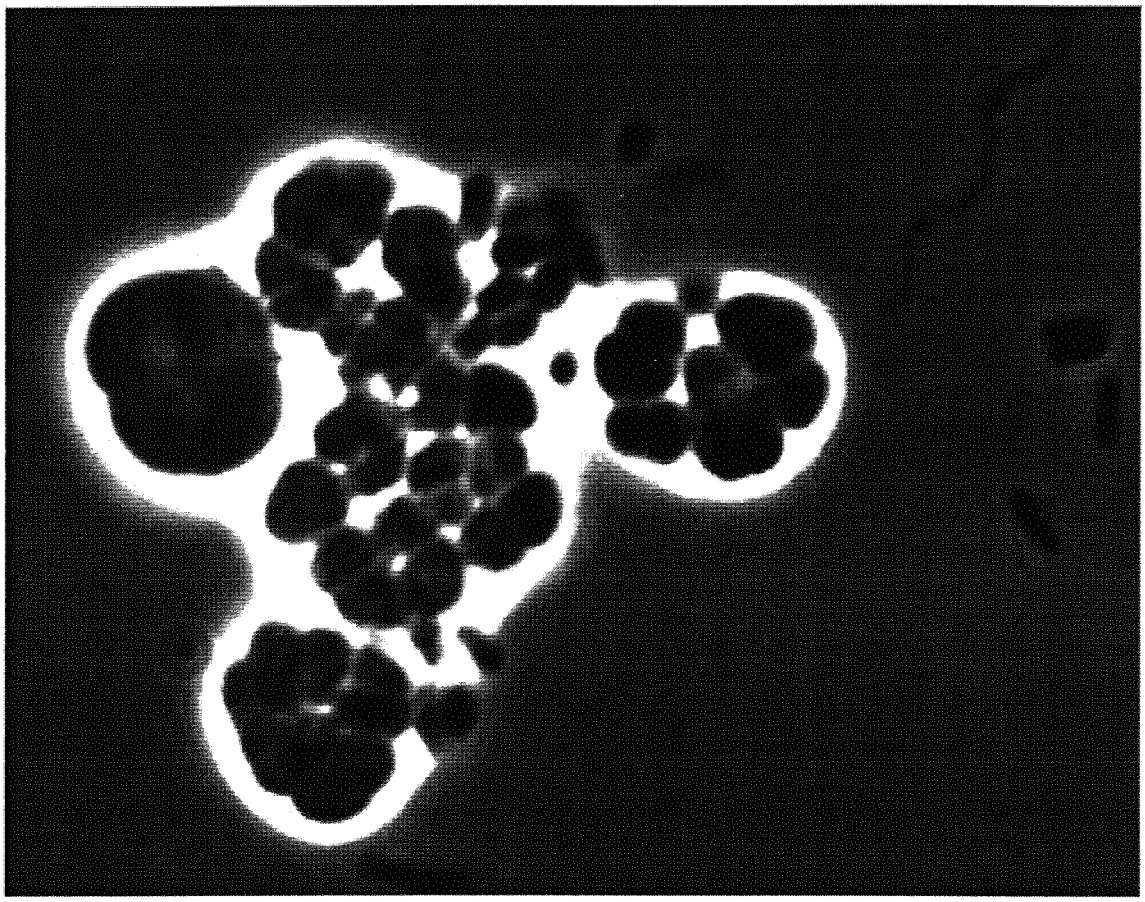


Figure 2: The larger *Methanosarcina*-type cells in Enrichment 5 amended with 2-methoxypropene.

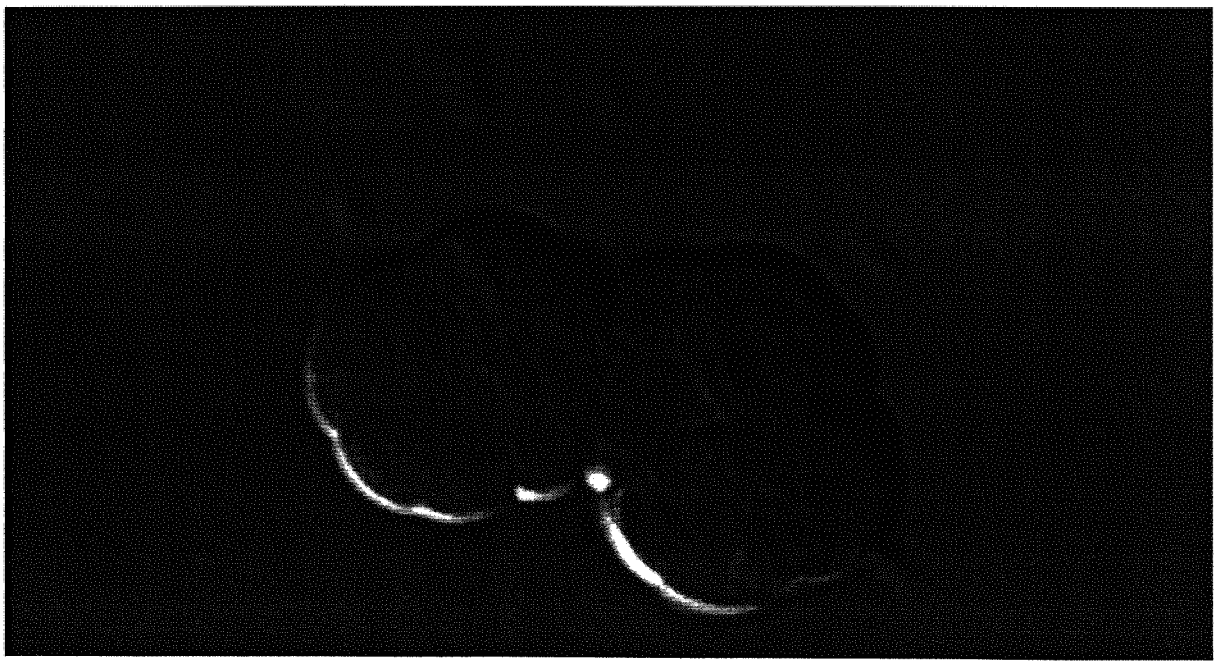


Figure 3: The larger *Methanosarcina*-type cells (left) and a protozoan (right) in Enrichment 5 amended with TMB.

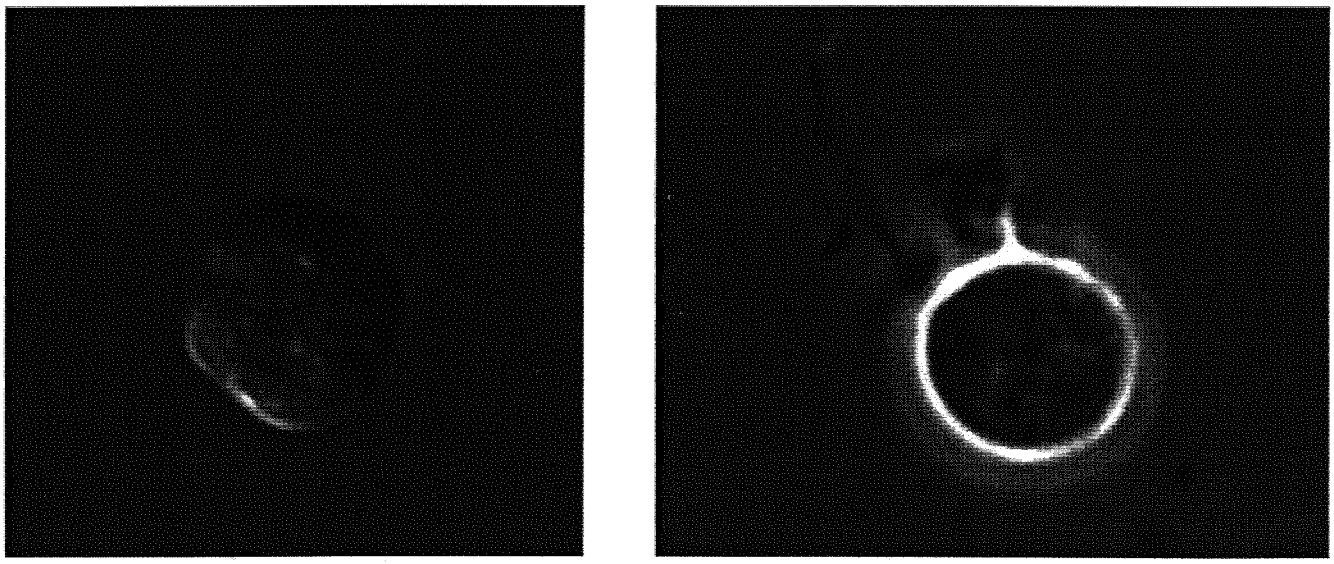


Figure 4: Consortia of several morphotypes in Enrichment 5 amended with TMB.

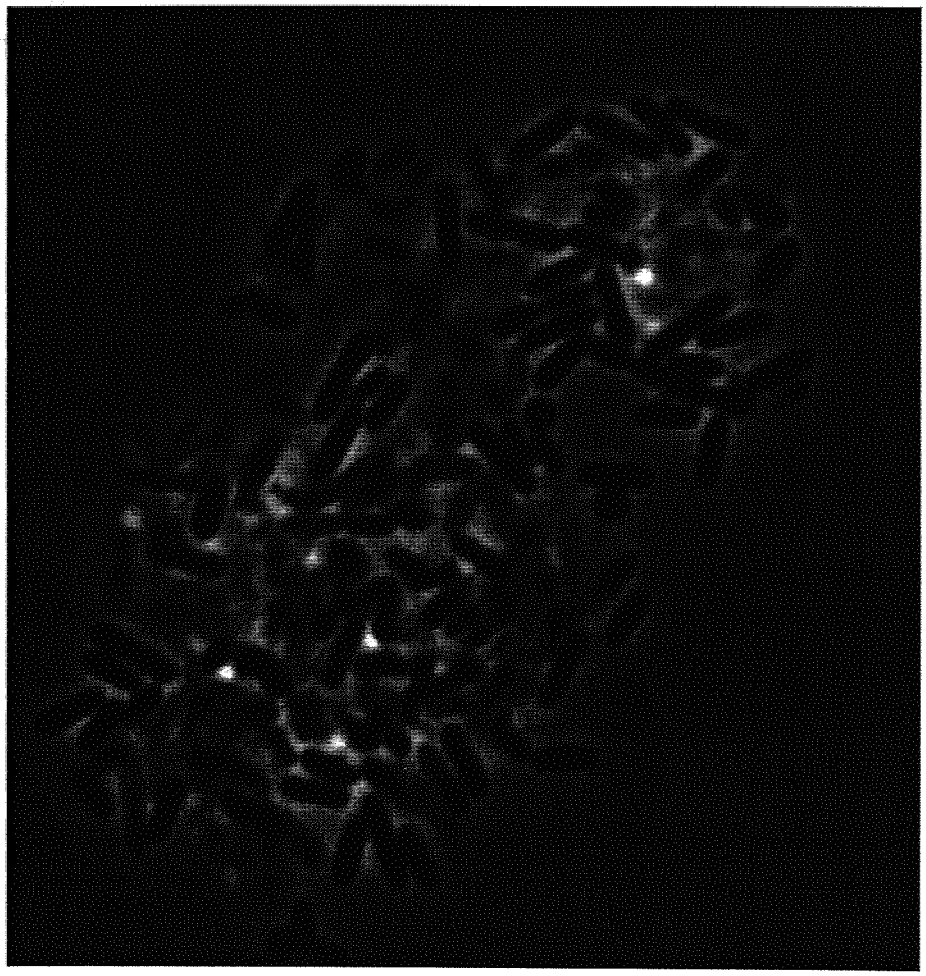


Figure 5: Small *Methanosarcina*-like cells in Enrichment 1 amended with TMB.

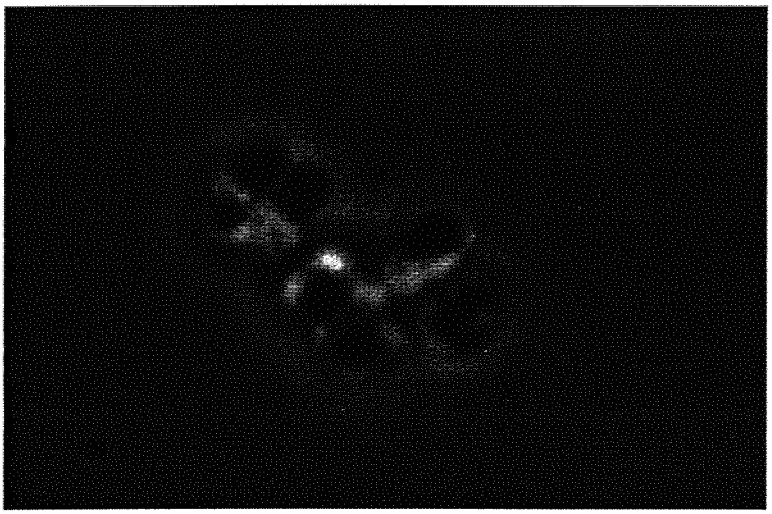


Figure 6: Consortia of several morphotypes in Enrichment 1 amended with TMB.

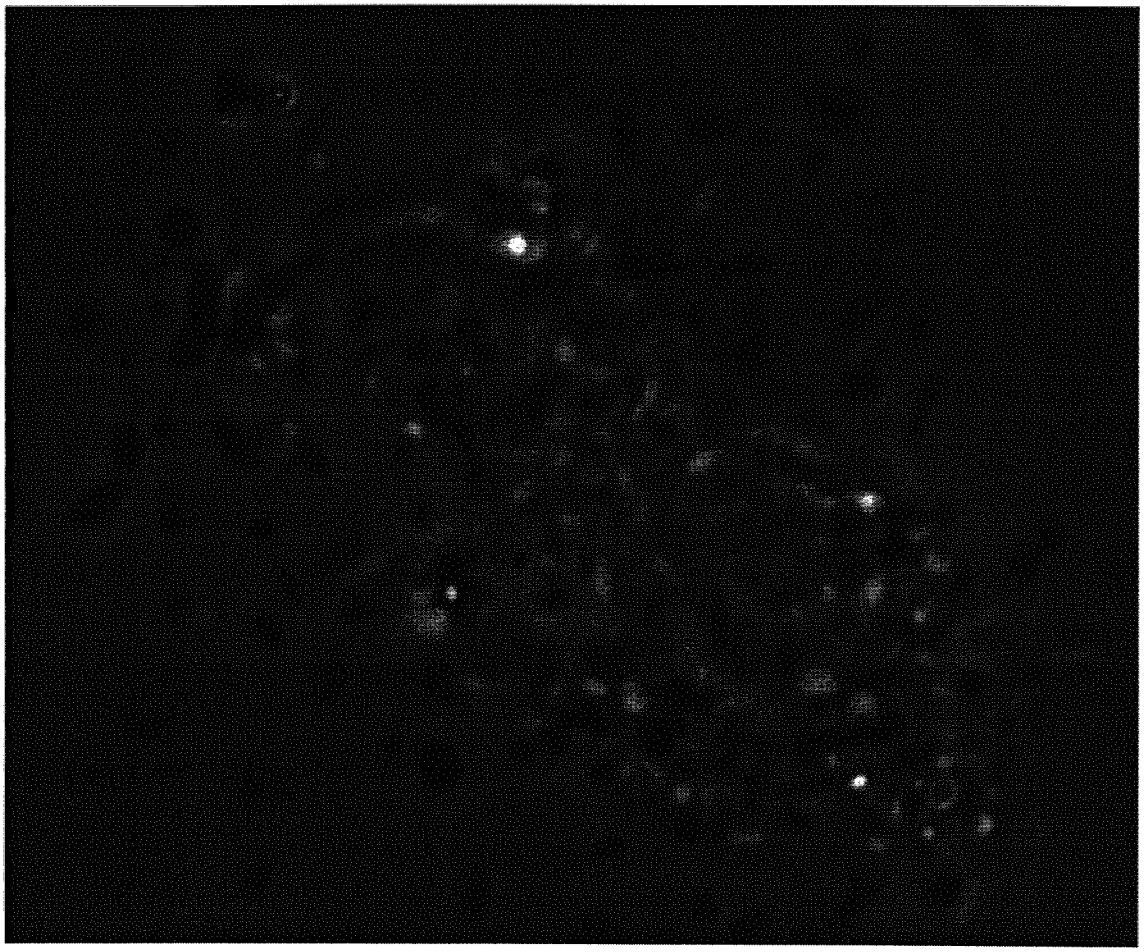


Figure 7: Several morphotypes, including the larger *Methanosarcina*-type cell, in Enrichment 1 amended with 2-methoxypropene.

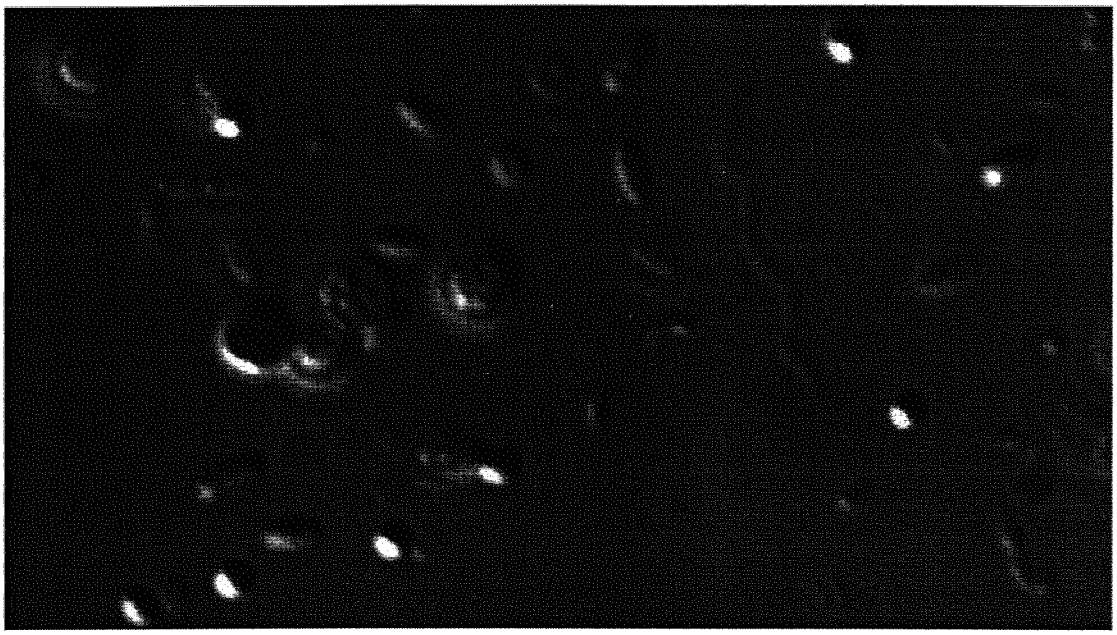
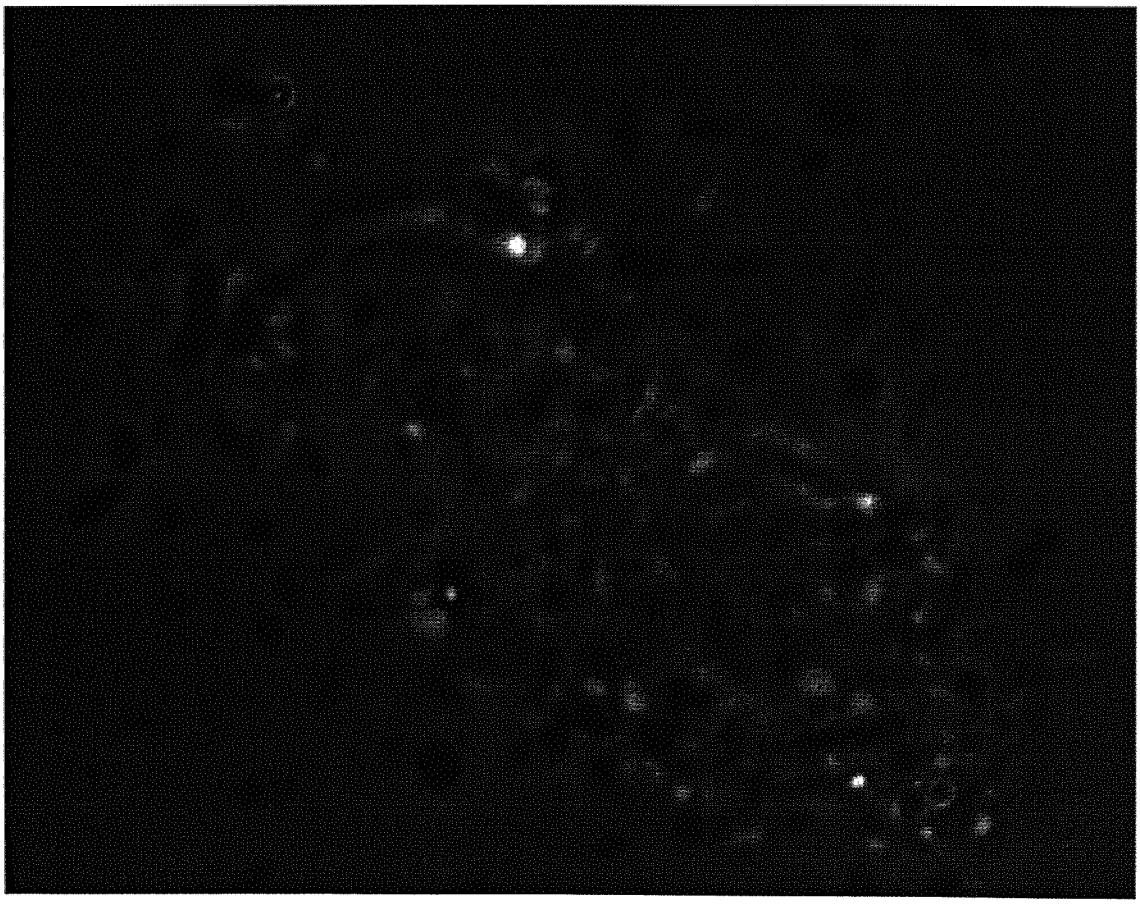


Figure 8: Clusters of morphotypes in Enrichment 1 amended with 2-methoxypropene.



Methanogenic Cultures

The results of the methanogenic studies suggest that the enrichment inoculated with the untreated septage is capable of utilizing tert-butyl alcohol (Table 3). The School Street Marsh enrichment had begun to produce methane from n-butanol, however, did not show significant methane production utilizing tert-butyl alcohol compared to the negative control. The tree soil did not produce methane under any treatment condition.

Table 3

Enrichment Sample Number	Treatment	Time-Zero % CH_4	6 Days % CH_4	12 Days % CH_4
1a*	Tert-butyl alcohol	0.012	0.16	0.44
1b*	Tert-butyl alcohol	0.014	0.046	0.14
1*	n-Butanol	0.013	0.215	0.57
1*	No substrate	0.014	0.04	0.14
2a+	Tert-butyl alcohol	0	0.006	0.02
2b+	Tert-butyl alcohol	0	0.039	0.16
2+	n-Butanol	0	0.073	1.02
2+	No substrate	0	0.007	0.03
3a#	Tert-butyl alcohol	0	0	0
3b#	Tert-butyl alcohol	0	0	0
3#	n-Butanol	0	0	0
3#	No substrate	0	0	0

*Untreated septage , +School Street Marsh, #Tree Soil

Discussion

The ability of the homoacetogenic enrichments to utilize 2-methoxypropene and trimethoxybenzoate, based on methane production and the presence of *Methanosarcina*-like archaea, suggests a link between acetogenesis and methanogenesis. For example, in the event that 2-methoxypropene is activated by homoacetogens to produce methanol and acetone, the methanol could be converted to acetate by the acetogens or to methane by methanogens. The acetone could be transformed to acetoacetate by a carboxylation reaction and then to 2 acetate molecules. The acetate could be further transformed to methane by methanogens. Such a scenario has been documented in the literature (Platen and Schink, 1987). A rough mass balance indicates that approximately 20% of the 2-methoxypropene was converted to methane by the subcultures of enrichment 5. Further analyses of intermediate products is necessary to propose a mechanism The enrichments that did not produce methane in the 2-methoxypropene and trimethoxybenzoate treatments (4 and 6) may have been inoculated with culture that was not viable or dying. It is important to note that lack of methane production in the other methoxy treatments (MTBE, 3-methoxycyclohexanecarboxylic acid, an 1,1,2-trimethoxyethane) does not necessarily mean there was no substrate transformation. It is possible that the homocetogens converted the substrates to acetate. Lack of acetate transformation to methane may indicate that the methanogens in the cultures were inhibited by the substrates. Further studies should focus on the measurement of acetate and other possible intermediates in order to calculate a rigorous mass balance. With regard to MTBE, such studies provide important insight for the application of bioremediation strategies

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Appendix

Freshwater Media

100x FW-Base for Freshwater Media (S-, C-, and N-free) (Add 10 mL to 1 L)

Water	1 Liter
NaCl	100 g
$MgCl_2$-$6H_2O$	40 g
$CaCl_2$-$2H_2O$	10 g
KH_2PO_4	20 g
KCl	50 g

100x Ammonium Chloride Solution for Freshwater or Marine Media (Add 10 mL to 1 L)

Water	1 Liter
NH4Cl	25 g

Sundry Solutions

1 M Sodium Bicarbonate, autoclaved under 100% CO_2 in serum bottles (Want 30 mM final concentration)

1 M MOPS, pH 7.2, filtered (want 5 mM final concentration so add 5 mL to 1 L)

1 M Sodium Sulfide, stored under N_2 at 4 °C (want 1 mM final concentration so add 1 mL to 1 L)

1000x Vitamin B_{12} Solution (Add 0.5 mL to 1 L)

Water	100 mL
Cyanocobalamin	100 mg

1000x 12-Vitamin Solution (Add 0.5 mL to 1 L)

Phosphate buffer, 10 mM pH 7.2	10 mL
Riboflavin	10 mg
Thiamine-HCl	100 mg
L-Ascorbic Acid	100 mg
D-Ca-pantothenate	100 mg
Folic acid	100 mg
Niacinamide	100 mg
Nicotinic acid	100 mg
4-Aminobenzoic acid	100 mg
Pyridoxine-HCl	100 mg
Lipoic acid	100 mg
NAD	100 mg
Thiamine pyrophosphate	100 mg

Titrate with NaOH until dissolved; Filter sterilize and freeze in 10 mL aliquots

1000x EDTA-Chelated Trace Elements Stock Solution (Add 1 mL to 1 L)

Water	987 mL
EDTA	5200 mg
Adjust pH to 6.0 with NaOH	

Add the following:

$FeSO_4$-$7H_2O$	2100 mg
H_3BO_3	30 mg
$MnCl_2$-$4H_2O$	100 mg
$CoCl_2$-$6H_2O$	190 mg
$NiCl_2$-$6H_2O$	24 mg
$CuCl_2$-$2H_2O$	2 mg
$ZnSO_4$-$7H_2O$	144 mg
Na_2MoO_4-$2H_2O$	36 mg
Sodium Vanadate	25 mg
Na_2SeO_3-$5H_2O$	6 mg
Na_2WO_4-$2H_2O$	8 mg

Filter sterilize

Methoxy Substrates:
Neutralized acids (p-methoxycyclohexanecarboxylic acid and p-methoxybenzoic acid) with equimolar amounts of NaOH.

HPLC Eluent:
0.3 mL of 60% perchloric acid (3 mM)

PROJECT 2: Chemotaxis Studies of Termite Gut Microbes

Abstract

The microbiota of the termite hindgut is highly diverse. A conspicuous group of bacteria observed in the hindgut is the spirochetes. This study attempted to observe spirochetes in the hindgut of *Reticulitermes flavipes* using chemotaxis assays. The substrates used as chemoattractants were: xylose, maltose, p-hydroxybenzoate, casamino acids and uric acid. The first assay design, the u-tube, did not result in chemotaxis of any type of microorganism to any of the substrates. The second assay design, flat capillary tubes inserted into an anaerobic suspension of cells, resulted in chemotaxis of fat rods to casmino acids and of spirochetes and rods to uric acid. Positive results appeared to depend on the preparation of the cell suspension and of the number of termite guts dissected.

Introduction

Termites exhibit a successful exploitation of lignocellulose as a food source due to their symbiotic interactions with microorganisms (Gastointestinal Microbiology, 1997). Termites feed on living, dead, decomposing, or highly decomposing plant matter (Wood, 1978). All termites have an anaerobic microbial community in the hindgut region of their alimentary tract. This community is highly diverse and can include prokaryotes, protozoa, bacteria and archaea. One of the most notable groups of bacteria observed in the termite gut is spirochetes (Breznak and Pankratz, 1977; To et al., 1978; Breznak 1984a, Czolij et al. 1985). The isolation of spirochetes has proven difficult. This study attempted to observe the spirochetes of termites using chemotaxis assays.

Chemotaxis is the movement of organisms toward a chemical. The chemotaxis assays were designed to provide substrates that spirochetes and other bacteria or archaea are exposed to in the termite gut. *Reticulitermes flavipes* is a common Eastern subterranean termite. Wood-feeding termites digest food that contains up to 100 times less N (on a dry-weight basis) than that exhibited by termite tissues (Breznak, 1984b) and have evolved symbiotic interactions with their gut microbes to sequester N. For this reason, casamino acids and uric acid were chosen as substrates that may serve as a nitrogen source. Uric acid can be fermented to acetate, CO_2 and NH_3 by anaerobic bacteria (Potrikus and Breznak, 1981). In addition to these substrates, xylose, maltose were selected as sugar sources, and para-hydroxybenzoate was selected because it is a monomer of lignin (complex aromatic polymer that is the major component of wood).

Methods

Preparation of chemoattractant and buffer solutions

Chemoattractant solutions were made by dissolving the substrates in a buffered saline solution (BSS) (10.8 mM K_2HPO_4, 6.9 mM KH_2PO_4, 21.5 mM KCl, 24.5 mM NaCl, 2 mM DTT (pH = 7.2). The buffer was autoclaved and cooled under N_2 before the DTT was added. The substrates were xylose (2 mM), maltose (2 mM), p-hyroxybenzoate (2 mM), casamino acids (2%) and uric acid (2 mM). The acids were neutralized with an equimolar amount of NaOH (except for the casamino acid solution). Solutions were filter-sterilized into autoclaved bottles.

BSS was also prepared with methyl cellulose (0.5% final concentration). The solution was autoclaved and methyl cellulose was added while it cooled (the solubility of methyl cellulose increases as the solution cools). DTT was also added the after the BSS was autoclaved.

The BSS with methyl cellulose was used for wet mounts because the microbiota need something viscous to move through and it keeps them alive for a longer period of time.

Dissection of the termite

Termites were collected in Woods Hole, MA. The gut of *Reticulitermes flavipes* was dissected on a microscope slide. Termites were placed in a glass petri dish with a wet chem-wipe. The petri dish was on ice to anaesthetize the termites. Termites were picked up by their heads and placed on a slide. Using fine-pointed tweezers, the end of the termite was "pulled" down until the hindgut was removed. The paunch was torn to release the gut fluid. For observation under the microscope a drop of BSS containing methyl cellulose was placed on the slide and a coverslip was placed on top.

Chemotaxis Assays

Design # 1.

A gut specimen was placed on a slide with a u-tube (See Appendix). The u-tube was covered with a microscope cover slip. The "chamber" created by the u-tube was filled with BSS buffer containing methyl cellulose (0.5 %). A thin capillary tube was prepared by sealing one end in a Bunsen burner flame. The hot open end was placed into the chemoattractant solution. The solution moved into the tube due to the gradient in temperature. The capillary tube was then placed into the chamber. Triplicates of each chemotaxis assay were prepared and observed over several hours. This experiment was also repeated using the gut fluid of the specimens without the gut tissue. The chemoattractants were xylose (2 mM), maltose (2 mM), p-hydroxybenzoate (2 mM), uric acid (2 mM), and casamino acids (2 %). The negative control was BSS.

Design #2

Several (6 to 12) gut specimens were placed on a slide. A drop of BSS (without methyl cellulose) was placed on the slide and a cell suspension was made and then diluted in an Eppendorf tube with 1 mL of BSS. A new slide was used for the assay (See Appendix). Two cover slips were broken in half and placed at the ends of the slide and sealed with vaspar. A long coverslip was placed on top and sealed with vaspar. The chamber was filled with the cell suspension and then sealed on one side with vaspar. Flat capillary tubes were placed in the chemoattractant solutions. The solutions moved into the tubes by capillary action. One end of the capillary tube was sealed with plasticine. The capillary tubes were placed in the chamber and then the remaining space between the tubes were sealed with vaspar. Each assay consisted of duplicate capillary tubes with the following substrates: casamino acids (2 %), uric acid (2 mM), and BSS (negative control).

Results

The results of the chemotaxis assays using design 1 did not result in chemotaxis for any of the tested substrates. The results of the chemotaxis assays using design 2 were positive for both casamino and uric acid (See Table 1) when 12 or more termites were dissected and suspended in a 1 mL buffered solution (results were negative for the assay that used the gut fluid of 6 termites). Relative to the negative control, the dominant morphology in the capillary tubes with casamino acids were rods (approximately 25 times greater than the control) (See Figure 1). No spirochetes or other morphologies were observed. In the uric acid capillaries, there was some

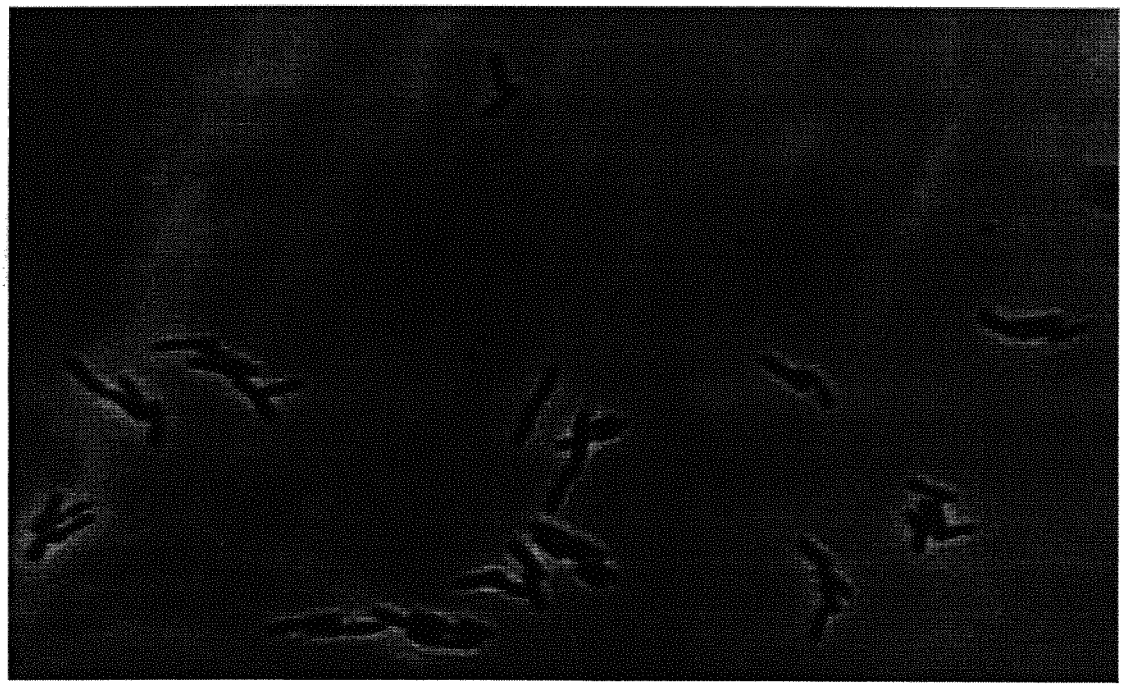
chemotaxis of the same type of rods observed in the casamino tubes but not relative to the control. Uric acid did result in chemotaxis of several types of spirochetes. The number of spirochetes was 4 times greater than the control. It was also observed that the rods formed clusters when they died in the tubes.

Table 1

Morphology	Casamino Acids	Uric Acid	BSS (Control)
Rods	25*	*	*
Spirochetes	-	4*	*
Other	-	*	*

*Indicates 1 unit in abundance
-No chemotaxis

Figure 1. Chemotaxis of rods in the flat capillary tubes containing casamino acids



Discussion

The negative results of the chemotaxis assays using design 1 may be attributed to several conditions. The most important condition may be due to the fact that the dissected gut was not suspended in the BSS solution. The microorganisms remained associated with the gut tissue despite the presence of the attractant solutions. Even when the gut fluid was used without the gut tissue, the sample was still a dense substance that did not disperse. An improvement of this method would involve making a suspension of cells and filling the chamber with the suspension.

It is also possible that the buffer solution did not stay anaerobic despite the DTT and the bacteria and archaea died.

The positive results of the chemotaxis assays using design 2 may be further evidence that design 1 was not satisfactory. It is important to note, however, that the diversity and abundance of gut microbes played an important role in these assays. Results were only positive when at least 12 termite guts were dissected. The next step in this project would be to try a broader range of substrates to

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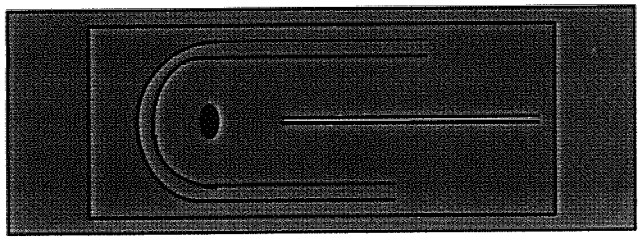
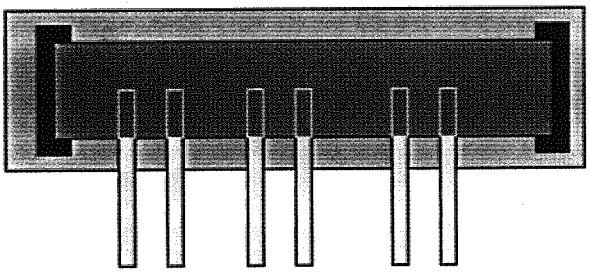
Appendix

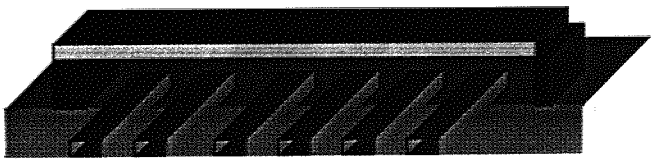
Design 1



Design 2



Plan-view



Side-view