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Treponema innocens Lipids and Further Description of an Unusual Galactolipid of *Treponema hyodysenteriae*

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The lipids of *Treponema innocens*, type strain B256, formerly considered a nonpathogenic isolate of *T. hyodysenteriae*, have been analyzed and compared with the lipids of *T. hyodysenteriae*. The lipids of *T. innocens* comprised 16% of the cell dry weight. Polar lipids amounted to about two-thirds of the total lipids and consisted of 61.9% phospholipids and 38.1% glycolipid. Neutral lipids consisted mainly of sterols. The phospholipids were principally phosphatidylglycerol, phosphatidylcholine, and cardiolipin. Minor amounts of lysophosphatidylcholine, sphingomyelin, and a relatively nonpolar, unidentified phospholipid were present. The latter lipid has not been detected in *T. hyodysenteriae*. The glycolipid fraction of *T. innocens* contained a single component, monoglucosyldiglyceride, in contrast to the occurrence in *T. hyodysenteriae* of two components: monogalactosyldiglyceride and a less-polar glycolipid tentatively identified as acylmonogalactosyldiglyceride (the additional acyl moieties being 86.8% acetyl, 11.6% propionyl, and 1.6% *n*-butyryl groups). Alk-1-enyl ether analogs comprised 24.6% of the total phospholipids and glycolipid of *T. innocens*, or about one-third of the amount in *T. hyodysenteriae*. The acyl and alk-1-enyl moieties of *T. innocens* consisted of ≥92% of 14:0, iso-15:0, and 16:0 chains. In contrast to *T. hyodysenteriae*, anteiso-15:0 moieties were not detected, and a reversed distribution of 14:0 and iso-15:0 alk-1-enyl moieties occurred in the two species.

Treponema hyodysenteriae, the primary agent in the etiology of swine dysentery (5, 11, 23, 25), is presently the only pathogenic *Treponema* sp. strain serially cultivated in vitro. Investigators in early studies observed that some isolates from swine differed in their capability to produce disease in specific-pathogen-free pigs (11, 23). Differences subsequently found in the DNA sequence homology of the pathogenic and nonpathogenic isolates suggested that each group might represent a distinct species (20). These findings in conjunction with differences in the ability of the two types of isolates to produce beta hemolysis, ferment fructose, and produce indole prompted Kinyon and Harris (10) in 1979 to propose a new species name, *T. innocens*, for the nonpathogenic strains.

We have reported that *T. hyodysenteriae*, pathogenic type strain B78, differs markedly from other *Treponema* sp. strains with regard to lipid composition and metabolism (18). The present study was undertaken to determine the lipid composition of a representative of the nonpathogenic species, *T. innocens*, type strain B256 (10), for comparison with *T. hyodysenteriae*. The lipids of this nonpathogenic organism were distinct from *T. hyodysenteriae* in several major characteristics. The unusual galactolipid described by us in *T. hyodysenteriae* B78 (18) has also been tentatively identified in this study and found to be absent from *T. innocens*.

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MATERIALS AND METHODS

Treponemes. *T. innocens* strain B256 and *T. hyodysenteriae* strains B204 and B78 were obtained from D. L. Harris, Iowa State University, Ames. The history and various characteristics of these isolates have been reported (10, 11, 20). Strains B256 and B204 were propagated and collected for lipid analyses between in vitro passages 16 and 19 as previously described (9, 18). The number of passages for strain B78 was unknown.

Lipid extraction and fractionation. Lipids were extracted from freshly harvested early-stationary-phase organisms (17) and purified by preparative thin-layer chromatography as previously described (18), except for the following modifications. The single glycolipid present in *T. innocens* migrated with a minor phospholipid in the solvent system of chloroform-methanol-28% ammonium hydroxide (60:15:2, vol/vol). These two lipids were separated by additional chromatography with the solvent system used previously for phospholipid fractionation. Lipids were eluted from the silica gel with methanol-chloroform-water (2:1:0.8, vol/vol) and recovered in the chloroform layer after adjusting the solvent ratio to 1:1:0.9 (vol/vol).

Analysis of lipids. Methods for the identification and quantitation of individual diacyl and alk-1-enylacyl lipids, fatty acids, dimethyl acetals, phosphorus, and sugars with thin-layer and gas-liquid chromatography have been described in detail by us in an earlier report (18).

Volatile fatty acid analysis. An unidentified and nonpolar galactolipid otherwise largely indistinguishable from monogalactosyldiglyceride has been described by us in *T. hyodysenteriae*, type strain B78 (18). To determine if the possible presence of short-chain, volatile fatty acids in this galactolipid might be the cause of the reduced polarity, alkaline hydrolysis of the isolated lipid was performed at 50°C for 60 min in 0.6 N NaOH in 90% methanol. The reaction mixture was then acidified with 35% H_3PO_4, and the fatty acids were analyzed with a Fisher-Victoreen 4000 gas-liquid chromatograph equipped with a flame ionization detector. A stainless steel column (183 by 0.32 cm) packed with 15% SP-1220–1% H_3PO_4 on 100/120 mesh Chromosorb W AW (Supelco, Inc., Bellefonte, Pa.) was used at 110°C. Isobutyric acid was employed as an internal standard, and the detector response was determined for individual fatty acids.

RESULTS

Identification and composition of the polar lipids. The lipid profile of *T. innocens* can be compared to that of *T. hyodysenteriae* B204 by use of Fig. 1. Lipids were tentatively identified with specific color reagents, and by comparison of mobilities in several solvent systems (18) with those of authentic standards and the lipids characterized earlier by us from *T. hyodysenteriae* B78. The types and quantitative composition of the lipids, including the acyl and alk-1-enyl groups, in *T. hyodysenteriae* B204 were similar to those of strain B78 (18) and will not be discussed in detail in this report.

The chromatograms (Fig. 1) indicate that *T. innocens* differed from *T. hyodysenteriae* with regard to components C and J. Component J, detected in *T. innocens* but not in *T. hyodysenteriae*, was a minor phospholipid that was not further characterized. Component C, a galactolipid found in *T. hyodysenteriae*, was absent in *T. innocens*. This lipid appeared to be a derivative of monogalactosyldiglyceride which contained a component attached to galactose that was not a long-chain fatty acid (18). Analysis of the acidified reaction products after alkaline deacylation of component C indicated that short-chain volatile fatty acids were present in the molecule in the proportions of 86.8% acetic, 11.6% propionic, and 1.6% *n*-butyric acids. Component C is therefore tentatively identified as acylmonogalactosyldiglyceride, the additional acyl group consisting of short-chain rather than

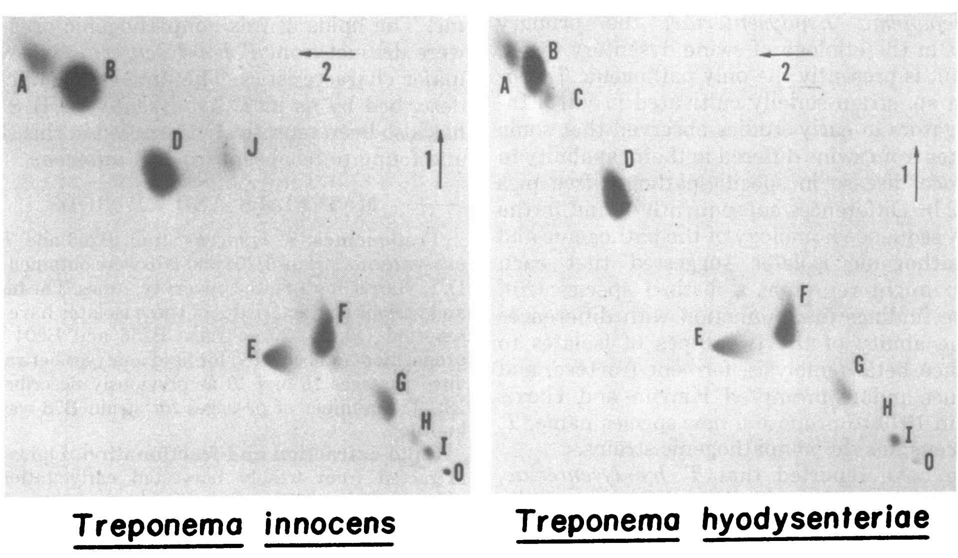

FIG. 1. *Two-dimensional thin-layer chromatograms on silica gel H of the total lipids of T. innocens B256 and T. hyodysenteriae B204. Lipids were extracted from early-stationary-phase cells grown anaerobically in Trypticase soy broth containing 10% fetal calf serum. Chromatograms were developed in direction 1 with chloroform-methanol-28% ammonium hydroxide (60:30:4, vol/vol) and developed in direction 2 with chloroform-methanol-water (65:24:4, vol/vol) before charring with 50% H_2SO_4. Identity of spots: A, neutral lipids; B, sterols; C, acylmonogalactosyldiglyceride (the added acyl moieties were short-chain rather than long-chain fatty acids); D, monoglycosyldiglyceride (the sugar moiety was 100% glucose in T. innocens and 100% galactose in T. hyodysenteriae); E, cardiolipin; F, phosphatidylglycerol; G, phosphatidylcholine; H, sphingomyelin; I, lysophosphatidylcholine; J, unidentified phospholipid; O, origin.*

long-chain fatty acids.

Component D from *T. innocens* had staining characteristics and mobility on silica gel H plates similar to those of monogalactosyldiglyceride from *T. hyodysenteriae* (18) and from plant tissue. The lipid from *T. innocens* (R_f, 0.24) migrated farther than did monogalactosyldiglyceride from *T. hyodysenteriae* (R_f, 0.12) or plant tissue (R_f, 0.13) on boric acid-containing silica gel G plates developed with chloroform-methanol-water-28% ammonium hydroxide (70:30:3:2, vol/vol). Gas-liquid chromatographic analysis of the trimethylsilyl derivatives of the methyl glycosides identified glucose as the only sugar in this lipid from *T. innocens*. Thus, component D was identified as monoglucosyldiglyceride in *T. innocens*. It is entirely monogalactosyldiglyceride in *T. hyodysenteriae* (18). Migration of these lipids on boric acid-containing plates differed in a manner comparable to gluco- and galactocerebrosides (8) due to the greater ability of galactose to form borate complexes.

Lipids of *T. innocens* amounted to 16% of the cellular dry weight and 6.0 mg/liter of culture. About one-third of the lipids were neutral lipids comprised mainly of sterols (Fig. 1) derived from the serum-containing medium. The phospholipids and glycolipids were quantitatively distributed as indicated in Table 1. Sphingomyelin occurred in trace amounts presumably originating from the medium.

TABLE 1. *Composition of the polar lipids of T. innocens B256 and proportion of the lipids in the alk-1-enylacyl form*

Lipid	% by wt of total polar lipids[a]	Mol% in alk-1-enylacyl form[b]
Phospholipid		
Phosphatidylglycerol	25.4	24.4
Phosphatidylcholine	16.2	3.9
Cardiolipin	13.0	18.3
Lysophosphatidylcholine	3.6	ND(−)
Unidentified phospholipid J	2.4	ND(+)
Sphingomyelin	1.4	ND(−)
Glycolipid		
Monoglucosyldiglyceride	38.1	40.5

[a] Quantitation was based on gravimetric determinations, phosphorus analyses, and acyl plus alk-1-enyl analyses by gas-liquid chromatography.

[b] Percentages of individual lipids existing in the alk-1-enylacyl form were calculated from the molar ratios of the alk-1-enyl chains, analyzed as dimethyl acetals, to the acyl groups, analyzed as methyl esters. Values represent moles of the alk-1-enylacyl form of the individual lipids per 100 mol of the total diacyl and alk-1-enylacyl forms. ND, Not determined; (+) positive or (−) negative reaction for aldehydes (plasmalogens) when sprayed with 2,4-dinitrophenylhydrazine in 2 N HCl.

Alk-1-enyl ether analogs. Significant proportions of the major phospholipids and the glycolipid of *T. innocens* existed in the alk-1-enylacyl form (Table 1). This form (also termed plasmalogen when referring to glycerophospholipids) contains an alk-1-enyl group attached by ether linkage at C-1 of glycerol instead of a fatty acid esterified at this position. Of the minor phospholipids, only phospholipid J yielded a positive reaction for plasmalogen on chromatographic plates. Alk-1-enylacyl forms of the individual lipids were much less abundant in *T. innocens* than in *T. hyodysenteriae*. The alk-1-enylacyl analogs comprised 24.6% of the total polar lipids of *T. innocens* compared with 75.5% in *T. hyodysenteriae* B78 (18) and 69.2% in *T. hyodysenteriae* B204 (data not shown).

Composition of acyl and alk-1-enyl moieties. The distributions of the acyl and alk-1-enyl groups of the lipids of *T. innocens* are given in Table 2. Saturated chains predominated. The only unsaturated acyl group detected, 18:1, and also the 18:0 moiety were concentrated in phosphatidylcholine and lysophosphatidylcholine. The major portion of the acyl moieties in the total lipids consisted of about equal amounts of iso-15:0 and 16:0, whereas iso-15:0 predominated among the alk-1-enyl groups. Although the types of acyl and alk-1-enyl moieties were practically the same as in *T. hyodysenteriae* (18), the two species differed in that anteiso-15:0, present in *T. hyodysenteriae*, was not detected in *T. innocens*. A reversed distribution of 14:0 and iso-15:0 alk-1-enyl moieties also existed between the organisms. The relative amounts of 14:0 and iso-15:0 alk-1-enyl moieties in the total lipids of *T. innocens* were 13 and 73% compared to 46 and 18%, respectively, in *T. hyodysenteriae* (18).

DISCUSSION

Like *T. hyodysenteriae* (18), the lipid composition and metabolism of *T. innocens* have been found in this study to differ remarkably from those of other *Treponema* (15). In addition, although the lipids of *T. innocens*, formerly classified as nonpathogenic *T. hyodysenteriae* (10), were similar to *T. hyodysenteriae* in many respects, several major distinguishing characteristics were noted in the lipids of the two species. The principal phospholipids of both organisms were phosphatidylglycerol, phosphatidylcholine, and cardiolipin. The only qualitative difference in the phospholipids was the presence of the minor unidentified phospholipid J in *T. innocens* that was not found in *T. hyodysenteriae*.

TABLE 2. *Relative percent composition of the acyl and alk-1-enyl moieties of the lipids of T. innocens B256*[a]

Moiety[b]	% Composition									
	Total lipids		Monoglucosyldiglyceride		Phosphatidylglycerol		Cardiolipin		Phosphatidylcholine	Lysophosphatidylcholine
	Acyl	Alkenyl	Acyl	Alkenyl	Acyl	Alkenyl	Acyl	Alkenyl	Acyl	Acyl
Unknown	–[c]	–	–	+	–	1	–	4	–	–
14:0	5	13	4	11	5	13	5	13	+	+
i-15:0	43	73	55	78	41	66	42	67	14	1
15:0	+	2	+	2	+	2	+	2	+	+
16:0	44	12	36	8	48	16	44	13	58	22
i-17:0	1	–	+	–	2	–	1	–	2	+
ai-17:0	+	–	+	–	1	–	+	–	1	+
17:0	+	–	+	–	+	–	+	–	1	2
18:0	4	–	–	–	2	1	5	–	17	53
18:1	1	–	–	–	+	–	+	–	5	20

[a] Acid methanolysis of the lipids was performed at 100°C for 2 h. Methyl esters of the fatty acids and dimethyl acetals of the alk-1-enyl moieties were separated by thin-layer chromatography with benzene before analyses by gas-liquid chromatography. Data on the alk-1-enyl compositions are presented only for the three principal alk-1-enyl ether lipids (Table 1).

[b] Number of carbon atoms:number of double bonds; i, iso branched; ai, anteiso branched.

[c] +, Amount less than 1%; –, not detected.

The glycolipids of *T. innocens* differed dramatically from *T. hyodysenteriae* as well as from other *Treponema*. Whereas both species contained monoglycosyldiglyceride, the sugar moiety was 100% glucose in *T. innocens* and 100% galactose in *T. hyodysenteriae* (18). Monoglycosyldiglyceride has been reported in all *Treponema* examined (15) except *T. pallidum* (17) and in all of the *Spirochaetales* (14–16, 19) except *Leptospira* (7). Galactose is usually present as the only sugar moiety, or galactose predominates in combination with glucose (15). Only in some *Spirochaeta* species is the sugar moiety exclusively glucose (15) as in *T. innocens*.

Another major difference in the glycolipids of *T. innocens* and *T. hyodysenteriae* was the presence only in *T. hyodysenteriae* of the unusual galactolipid (18) tentatively identified in this report as primarily the acetyl ester of monogalactosyldiglyceride. This lipid has not been found in any other spirochete. Clarke et al. (2) have described a similar lipid in *Butyrivibrio* which was largely the *n*-butyryl ester of monogalactosyldiglyceride. Acyl esters of monoglycosyldiglycerides have been reported by Veerkamp (24) in *Bifidobacterium*, but these contain long-chain rather than short-chain fatty acids.

Significant amounts of the lipids of *T. innocens* existed in the alk-1-enylacyl (plasmalogen) form similar to other anaerobic bacteria (see reference 18 and references therein). The alk-1-enylacyl form of the glycolipid is rather unusual, being reported previously in *Butyrivibrio* (2) and in *T. hyodysenteriae* (18). Among spirochetes, only *T. hyodysenteriae* and *Treponema phagedenis* are known to contain alk-1-enyl ether lipids (18). The three major lipids of *T. hyodysenteriae* exist in the alk-1-enylacyl form (74.8 to 96.4%), whereas in *T. phagedenis* only the choline phospholipid is found in this form (20%). The alk-1-enyl ether lipids of *T. innocens* were present in only about one-third the amounts observed in *T. hyodysenteriae*. This is interesting in view of the similarity of these two organisms in morphology, natural habitat, metabolism, and genetics (10, 20).

Although the acyl and alk-1-enyl moieties of *T. innocens* were similar to those of *T. hyodysenteriae* (18), the organisms could be differentiated by the presence of anteiso-15:0 moieties only in *T. hyodysenteriae* and by the ratio of 14:0 to iso-15:0 alk-1-enyl moieties, the latter predominating in *T. innocens*. The marked dissimilarity of the acyl and alk-1-enyl groups of *T. innocens* and those of the serum-containing medium (18) indicated that the organism can synthesize long-chain fatty acids like *T. hyodysenteriae* (18) and also, interestingly, like the *Spirochaeta* (15). In this regard, *T. innocens* and *T. hyodysenteriae* differ remarkably from most *Treponema* which are unable to synthesize long-chain fatty acids (6, 19). They also appear to differ from oral and intestinal spirochetes that require short-chain fatty acids for growth and presumably for the synthesis of long-chain fatty acids (3, 4, 16, 21, 22). Serum is usually added to medium to supply the long-chain fatty acids required for growth of most *Treponema* (15).

Our results indicate that serum apparently does not perform this function in the growth of *T. innocens* and *T. hyodysenteriae*. Recent evidence suggests that a role of serum may be to provide sterols required for growth (13).

The definitive criterion for the differentiation of *T. innocens* and *T. hyodysenteriae* is enteropathogenicity in swine. A more practical approach is generally to distinguish the species by the degree of beta-hemolysis produced on blood agar plates (10). Tests for indole production and fructose fermentation may also be useful, but results are variable (10) and thus of questionable reliability. Recently, a growth-inhibition test (12) and isolation of a species-specific antigen (1) for *T. hyodysenteriae* have been described which may prove beneficial in identification of the organism and in the diagnosis of swine dysentery. Our results indicate that differences in the lipid compositions, particularly the glycolipids, of nonpathogenic *T. innocens* and pathogenic *T. hyodysenteriae* may also be exploited for these purposes.

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LITERATURE CITED

1. **Baum, D. H., and L. A. Joens.** 1979. Partial purification of a specific antigen of *Treponema hyodysenteriae*. Infect. Immun. **26**:1211-1213.
2. **Clarke, N. G., G. P. Hazlewood, and R. M. C. Dawson.** 1976. Novel lipids of *Butyrivibrio spp.* Chem. Phys. Lipids **17**:222-232.
3. **Cwyk, W. M., and E. Canale-Parola.** 1979. *Treponema succinifaciens* sp. nov., an anaerobic spirochete from the swine intestine. Arch. Microbiol. **122**:231-239.
4. **Hardy, P. H., Jr., and C. O. Munro.** 1966. Nutritional requirements of anaerobic spirochaetes. I. Demonstration of isobutyrate and bicarbonate as growth factors for a strain of *Treponema microdentium*. J. Bacteriol. **91**:27-32.
5. **Harris, D. L., R. D. Glock, C. R. Christensen, and J. M. Kinyon.** 1972. Swine dysentery. I. Inoculation of pigs with *Treponema hyodysenteriae* (new species) and reproduction of the disease. Vet. Med. Small Anim. Clin. **67**:61-64.
6. **Johnson, R. C., B. P. Livermore, H. M. Jenkin, and L. Eggebraten.** 1970. Lipids of *Treponema pallidum* Kazan 5. Infect. Immun. **2**:606-609.
7. **Johnson, R. C., B. P. Livermore, J. K. Walby, and H. M. Jenkin.** 1970. Lipids of parasitic and saprophytic leptospires. Infect. Immun. **2**:286-291.
8. **Kean, E. L.** 1966. Separation of gluco- and galacto- cerebrosides by means of borate thin-layer chromatography. J. Lipid Res. **7**:449-452.
9. **Kinyon, J. M., and D. L. Harris.** 1974. Growth of *Treponema hyodysenteriae* in liquid medium. Vet. Rec. **95**: 219-220.
10. **Kinyon, J. M., and D. L. Harris.** 1979. *Treponema innocens*, a new species of intestinal bacteria, and emended description of the type strain of *Treponema hyodysenteriae* Harris et al. Int. J. Syst. Bacteriol. **29**: 102-109.
11. **Kinyon, J. M., D. L. Harris, and R. D. Glock.** 1977. Enteropathogenicity of various isolates of *Treponema hyodysenteriae*. Infect. Immun. **15**:638-646.
12. **Lemcke, R. M., and M. R. Burrows.** 1979. A disc growth-inhibition test for differentiating *Treponema hyodysenteriae* from other intestinal spirochaetes. Vet. Rec. **104**:548-551.
13. **Lemcke, R. M., and M. R. Burrows.** 1980. Sterol requirement for the growth of *Treponema hyodysenteriae*. J. Gen. Microbiol. **116**:539-543.
14. **Livermore, B. P., R. F. Bey, and R. C. Johnson.** 1978. Lipid metabolism of *Borrelia hermsi*. Infect. Immun. **20**:215-220.
15. **Livermore, B. P., and R. C. Johnson.** 1974. Lipids of the *Spirochaetales*: comparison of the lipids of several members of the genera *Spirochaeta*, *Treponema*, and *Leptospira*. J. Bacteriol. **120**:1268-1273.
16. **Livermore, B. P., and R. C. Johnson.** 1975. The lipids of four unusual non-pathogenic host-associated spirochetes. Can. J. Microbiol. **21**:1877-1880.
17. **Matthews, H. M., T.-K. Yang, and H. M. Jenkin.** 1979. Unique lipid composition of *Treponema pallidum* (Nichols virulent strain). Infect. Immun. **24**:713-719.
18. **Matthews, H. M., T.-K. Yang, and H. M. Jenkin.** 1980. Alk-1-enyl ether phospholipids (plasmalogens) and glycolipids of *Treponema hyodysenteriae*. Analysis of acyl and alk-1-enyl moieties. Biochim. Biophys. Acta **618**: 273-281.
19. **Meyer, H., and F. Meyer.** 1971. Lipid metabolism in the parasitic and free-living spirochetes *Treponema pallidum* (Reiter) and *Treponema zuelzerae*. Biochim. Biophys. Acta **231**:93-106.
20. **Miao, R. M., A. H. Fieldsteel, and D. L. Harris.** 1978. Genetics of *Treponema*: characterization of *Treponema hyodysenteriae* and its relationship to *Treponema pallidum*. Infect. Immun. **22**:736-739.
21. **Smibert, R. M., and R. L. Claterbaugh, Jr.** 1972. A chemically defined medium for *Treponema* strain PR-7 isolated from the intestine of a pig with swine dysentery. Can. J. Microbiol. **18**:1073-1078.
22. **Socransky, S. S., W. J. Loesche, C. Hubersak, and J. B. MacDonald.** 1964. Dependency of *Treponema microdentium* on other oral organisms for isobutyrate, polyamines, and controlled oxidation-reduction potential. J. Bacteriol. **88**:200-209.
23. **Taylor, D. J., and T. J. L. Alexander.** 1971. The production of dysentery in swine by feeding cultures containing a spirochaete. Br. Vet. J. **127**:58-61.
24. **Veerkamp, J. H.** 1972. Biochemical changes in *Bifidobacterium bifidum* var. *pennsylvanicus* after cell wall inhibition. V. Structure of the galactosyldiglycerides. Biochim. Biophys. Acta **273**:359-367.
25. **Whipp, S. C., I. M. Robinson, D. L. Harris, R. D. Glock, P. J. Matthews, and T. J. L. Alexander.** 1979. Pathogenic synergism between *Treponema hyodysenteriae* and other selected anaerobes in gnotobiotic pigs. Infect. Immun. **26**:1042-1047.