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, phos-
phtidylinositol, 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, monogalactosyldiglyceride,
in contrast to the occurrence in T. hyodysenteriae of two components: monoga-
lactosyldiglyceride and a less-polar glycolipid tentatively identified as acylmono-
galactosyldiglyceride (the additional acyl moieties being 86.8% acetyl, 11.6% propionyl,
and 1.6% n-butyryl groups). Alk-1-enz 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-enz moieties of T. innocens
consisted of ≥92% of 14:0, isо-15:0, and 16:0 chains. In contrast to T. hyodysen-
teriae, antiseo-15:0 moieties were not detected, and a reversed distribution of 14:0
and iso-15:0 alk-1-enz 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 Trepo-
nema sp. strain serially cultivated in vitro. In-
vestigators 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 non-
pathogenic species, T. innocens, type strain
B256 (10), for comparison with T. hyodysenter-

ia. 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.

MATERIALS AND METHODS

Treponemes. T. innocens strain B256 and T. hy-
odysenteriae 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 un-
known.

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 previ-
ously for phospholipid fractionation. Lipids were
eluted from the silica gel with methanol-chloroform-
water (2:1:0.8, vol/vol) and recovered in the chloro-
form layer after adjusting the solvent ratio to 1:1:0.9
(vol/vol).

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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% H3PO4, 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% H3PO4 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 deacetylation 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% H2SO4. 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, phosphatidylylycerol; G, phosphatidylcholine; H, sphingomyelin; I, lysophosphatidylcholine; J, unidentified phospholipid; O, origin.](http://jb.asm.org/)

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long-chain fatty acids.

Component D from \textit{T. innocens} had staining characteristics and mobility on silica gel H plates similar to those of monogalactosyldiglyceride from \textit{T. hyodysenteriae} (18) and from plant tissue. The lipid from \textit{T. innocens} \((R_f\, 0.24)\) migrated farther than did monogalactosyldiglyceride from \textit{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 \((70:30:3:2,\ \text{vol/vol})\). Gas-liquid chromatographic analysis of the trimethylsilyl derivatives of the methyl glycosides identified glucose as the only sugar in this lipid from \textit{T. innocens}. Thus, component D was identified as monoglucosyldiglyceride in \textit{T. innocens}. It is entirely monogalactosyldiglyceride in \textit{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 \textit{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 \((\text{Fig. 1})\) 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.

**Alk-1-enyl ether analogs.** Significant proportions of the major phospholipids and the glycolipid of \textit{T. innocens} existed in the alk-1-enylacyl form (Table 1). This form \((\text{also termed plasmalogen when referring to glycerophospholipids})\) 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 \textit{T. innocens} than in \textit{T. hyodysenteriae}. The alk-1-enylacyl analogs comprised 24.6% of the total polar lipids of \textit{T. innocens} compared with 75.5% in \textit{T. hyodysenteriae} \(B\) \(78\) \((18)\) and 69.2% in \textit{T. hyodysenteriae} \(B\) \(204\) (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 \textit{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 phosphatidylycholine and lysophosphatidylycholine. 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 \textit{T. hyodysenteriae} \((18)\), the two species differed in that anteiso-15:0, present in \textit{T. hyodysenteriae}, was not detected in \textit{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 \textit{T. innocens} were 13 and 73% compared to 46 and 18%, respectively, in \textit{T. hyodysenteriae} \((18)\).

**DISCUSSION**

Like \textit{T. hyodysenteriae} \((18)\), the lipid composition and metabolism of \textit{T. innocens} have been found in this study to differ remarkably from those of other \textit{Treponema} \((15)\). In addition, although the lipids of \textit{T. innocens}, formerly classified as nonpathogenic \textit{T. hyodysenteriae} \((10)\), were similar to \textit{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 phosphatidylycholine, phosphatidylcholine, and cardiolipin. The only qualitative difference in the phospholipids was the presence of the minor unidentified phospholipid J in \textit{T. innocens} that was not found in \textit{T. hyodysenteriae}.
The glycolipids of *T. innocens* differed dramatically from *T. hydysenteriae* 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. hydysenteriae* (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. hydysenteriae* was the presence only in *T. hydysenteriae* 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 *Butyrylobrio* 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 (plasmalogens) 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 *Butyrylobrio* (2) and in *T. hydysenteriae* (18). Among spirochetes, only *T. hydysenteriae* and *Treponema phagedenis* are known to contain alk-1-enyl ether lipids (18). The three major lipids of *T. hydysenteriae* 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. hydysenteriae*. 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. hydysenteriae* (18), the organisms could be differentiated by the presence of anteiso-15:0 moieties only in *T. hydysenteriae* 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. hydysenteriae* (18) and also, interestingly, like the *Spirochaeta* (15). In this regard, *T. innocens* and *T. hydysenteriae* 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).

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**Table 2. Relative percent composition of the acyl and alk-1-enyl moieties of the lipids of *T. innocens* B256a**

<table>
<thead>
<tr>
<th>Moiety</th>
<th>Total lipids</th>
<th>Monoglycosyldiglyceride</th>
<th>Phosphatidylglycerol</th>
<th>Cardiolipin</th>
<th>Phosphatidylcholine</th>
<th>Lyso-phosphatidylcholine</th>
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<tbody>
<tr>
<td></td>
<td>Acyl</td>
<td>Alkenyl</td>
<td>Acyl</td>
<td>Alkenyl</td>
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<tr>
<td>Unknown</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>1</td>
</tr>
<tr>
<td>14:0</td>
<td>5</td>
<td>13</td>
<td>4</td>
<td>11</td>
<td>5</td>
<td>13</td>
</tr>
<tr>
<td>i-15:0</td>
<td>43</td>
<td>73</td>
<td>55</td>
<td>78</td>
<td>41</td>
<td>66</td>
</tr>
<tr>
<td>15:0</td>
<td>+</td>
<td>2</td>
<td>+</td>
<td>2</td>
<td>+</td>
<td>2</td>
</tr>
<tr>
<td>16:0</td>
<td>44</td>
<td>12</td>
<td>36</td>
<td>8</td>
<td>48</td>
<td>16</td>
</tr>
<tr>
<td>i-17:0</td>
<td>1</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>2</td>
<td>-</td>
</tr>
<tr>
<td>ai-17:0</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>1</td>
<td>-</td>
</tr>
<tr>
<td>17:0</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>18:0</td>
<td>4</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>18:1</td>
<td>1</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
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</table>

a Acid methanolyis 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.
Our results indicate that serum apparently does not perform this function in the growth of *T. innocens* and *T. hydysenteriae*. 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. hydysenteriae* 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. hydysenteriae* 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. hydysenteriae* may also be exploited for these purposes.

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LITERATURE CITED


