

Inhibition of *Giardia intestinalis* by Extracellular Factors from Lactobacilli: an In Vitro Study

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The aim of the present work was to evaluate the effect of spent culture supernatants of different strains of lactobacilli on giardia trophozoites. The growth of *Giardia intestinalis* strain WB, as well as the attachment to the human intestinal epithelial cell line Caco-2, was evaluated by using proliferation and adhesion assays with radiolabeled parasites. In addition, scanning electron microscopy and flow cytometric analysis were performed. The effect of spent culture supernatants from lactobacilli was strain dependent. *Lactobacillus johnsonii* La1 significantly inhibited the proliferation of *G. intestinalis* trophozoites. Although the effect was strongly pH dependent, it was not simply due to lactic acid. According to flow cytometric analysis, trophozoites were arrested in G₁ phase but neither significant necrosis nor apoptosis could be detected. Bacterial cells or their spent culture supernatants were unable to modify trophozoite attachment to Caco-2 cells. However, trophozoites treated with spent culture supernatants had little, if any, proliferative capacity. These results suggest that La1 produces some substance(s) able to inhibit proliferation of *Giardia* trophozoites. Partial characterization of the factors involved in the anti-giardiasis action showed that they have a low molecular mass and are inactivated by heating. On this basis, it seems worthwhile to explore how colonization of the proximal small bowel with these lactic acid bacteria could interfere with giardiasis in vivo.

Giardia intestinalis is a flagellated protozoan which causes diarrheal disease worldwide. Its life cycle consists of two stages, cysts and trophozoites, which are adapted to survive in very diverse and hostile environments (16). Cysts are dormant, quadrinucleated structures responsible for the transmission of giardiasis. They are eliminated with feces and tolerate extreme conditions of temperature, pH, and tonicity. Transmission to mammals takes place mainly through contaminated water and foods (12). After ingestion, the sequential exposure to gastric acid and digestive enzymes promotes the transformation of cysts into trophozoites. They are binucleated, highly dividing, half-pear-shaped cells (ca. 10 µm in size) with a ventral adhesive disk that, together with four pairs of laterally arranged flagella, is involved in the attachment to the small intestinal epithelium (12). Trophozoites may persist in the small intestine for weeks to years. If they are carried downstream by the intestinal fluid, they normally encyst.

In children, infections may result in severe diarrhea, malabsorption, and failure to thrive (11). In contrast, approximately one-half of infected people, children and adults, remain asymptomatic (13). To date, no toxin has been isolated and no single virulence factor or mechanism has been proposed to explain the pathogenesis of the disease (1, 8, 13, 19).

Giardiasis is generally treated with antibiotics (e.g., nitroimidazoles and nitrofurans); however, in areas where it is endemic the emergence of resistant strains has led to a high rate of clinical failures (20).

It is widely recognized that resident microflora of the intes-

tine plays a role in the protection of the host against gut colonization by pathogens (30). Different mechanisms may be responsible for the microflora protective role: (i) specific competition for pathogen receptor sites on the mucosal surface, (ii) a nonspecific steric hindrance that constitutes a barrier for the pathogen access to the mucosal surface, (iii) production of antimicrobial products, (iv) competition for nutritional substrates, and (v) enhancement of the host's innate and adaptive immune responses. Nutritional strategies have been proposed to increase the components of the intestinal microflora that are more clearly associated with the above-mentioned protective activities (6). Interestingly, the studies on the protection afforded by the intestinal microflora or probiotics have been performed mainly with bacterial enteropathogens (4, 9). Recently, it has been suggested that the composition of the intestinal microflora may also influence the degree of parasitic infection (29). Therefore, an alternative approach to control giardia infection may result from the use of adherent, non-pathogenic lactic acid bacteria (LAB) to antagonize the attachment and proliferation of the trophozoites.

There is a large body of evidence showing that LAB can modulate the intestinal mechanisms of defense against enteropathogens in "in vivo" models (4, 5, 17, 26, 28). Since orally administered probiotic bacteria can become an important part of the proximal small bowel microflora, it is possible that these bacteria modify the intestinal environment through their metabolic activity. Moreover, the capacity of some probiotic strains to intimately associate with intestinal epithelial cells may modify the apical cell microenvironment where most pathogens attach and colonize the host. In giardiasis, attachment of the trophozoites to epithelial cells, followed by active proliferation, is required for progression of the disease.

Given that factors such as biliary secretions, organic acids,

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and other substances present in the intestinal lumen play an important role in the cell cycle of the parasite (15, 23), we studied the effect of extracellular factors from intestinal lactobacilli on strain WB of *G. intestinalis*.

MATERIALS AND METHODS

Bacterial cultures. *Lactobacillus acidophilus* NCC 2581, NCC 2538, and NCC 2592 isolated from cat feces, *L. acidophilus* NCC 2603, NCC 2613, and NCC 2628 isolated from dog feces, as well as the *L. johnsonii* La1 NCC 533, all from the NESTEC culture collection (Lausanne, Switzerland) were used. Microorganisms were grown in MRS broth (10) or modified TYI-S-33 medium (MTYI) which contained the following (per liter): casein digest (Difco, Detroit, Mich.), 20 g; yeast extract (BBL, Cockeysville, Md.), 10 g; dextrose (Merck, Darmstadt, Germany), 20 g; NaCl (Merck), 2 g; L-cysteine HCl (Sigma, St. Louis, Mo.), 2 g; ascorbic acid sodium salt (Merck), 0.2 g; K₂HPO₄ (Merck), 1 g; KH₂PO₄ (Merck), 0.6 g; and Tween 80, 1 g. The pH was adjusted to pH 7.0 with 5 N NaOH prior to sterilization through a 0.22- μ m (pore-size) filter.

Bacteria were reactivated twice before each experiment. Cultures were incubated for 16 h at 37°C in anaerobic conditions (GasPack Plus; BBL) and then centrifuged at 4,000 \times g for 10 min. The supernatants were removed, and the pH was adjusted to 6.0 or 7.0 with 5 N NaOH. Fresh culture medium, acidified with lactic acid to the same pH as that of bacterial cultures and then neutralized at pH 6.0 or pH 7.0, served as a control. In some experiments, the bacterial pellet was washed and resuspended in the culture medium to perform parasite adhesion assays in the presence of living lactobacilli.

Parasites. *G. intestinalis* strain WB (ATCC 30957) was purchased from the American Type Culture Collection (Rockville, Md.). Trophozoites were grown in Keister's modified TYI-S-33 medium (22), which contained the following (per liter): casein digest (Difco), 20 g; yeast extract (BBL), 10 g; dextrose (Merck), 10 g; bovine bile (Difco), 0.75 g; NaCl (Merck), 2 g; L-cysteine HCl (Sigma), 2 g; ascorbic acid sodium salt (Fluka, Buchs, Switzerland), 0.2 g; K₂HPO₄ (Merck), 1 g; KH₂PO₄ (Merck), 0.6 g; ferric ammonium citrate (Sigma), 22.8 mg; and adult bovine serum (Sigma), 100 ml. The medium also contained 15 ml of a solution composed of 1,000 IU of penicillin and 1,000 g of streptomycin (Gibco-BRL/Life Technologies, Paisley, Scotland)/ml. The pH was adjusted to pH 6.9 with 5 N NaOH prior to sterilization with a 0.22- μ m (pore-size) filter.

Parasites were cultured in polystyrene tissue culture flasks (LUX; Miles Laboratories, Inc., Naperville, Ill.) containing 40 ml of culture medium. Subcultures were performed by discarding the supernatant along with nonattached parasites, adding 5 ml of ice-cold culture medium, incubation in an ice-water bath for 10 min to dislodge adherent trophozoites, and inoculation of 0.2 ml of the resulting suspension into fresh medium. Incubations were performed at 37°C in the dark.

Adhesion assays. Parasites were radiolabeled by incubation with 2.5 Ci of [2-³H]adenine (21 Ci/mmol, 1 mCi/ml; Amersham Pharmacia Biotech, Buckinghamshire, United Kingdom)/ml at 37°C for 48 h. Caco-2 cells (passages 50 to 55) were grown in Dulbecco modified Eagle medium (AMIMED, Bioconcept, Allschwil, Switzerland) supplemented with nonessential amino acids (Gibco-BRL/Life Technologies), penicillin (12 IU/ml), streptomycin (12 μ g/ml), gentamicin (47 μ g/ml), and heat-inactivated 20% (vol/vol) fetal calf serum (AMIMED, Bioconcept). Monolayers were prepared in six-well tissue culture plates (Corning Glass Works, Corning, N.Y.) by seeding 2 \times 10⁵ cells per well and culturing them at 37°C in a 10% CO₂-air incubator. Culture medium was changed every 2 days. Adhesion assays were performed by using monolayers which had been cultured for 21 days.

One-hundred microliters of parasite suspension (10⁵ trophozoites, 53,000 \pm 3,000 cpm) was inoculated into either 1 ml of bacterial culture supernatant or its control and then incubated at 37°C for 1 h. Thereafter, adherent parasites were detached by incubating tubes at 0°C for 10 min. Then, 1 ml of this suspension was mixed with 1 ml of GKN solution (NaCl, 8 g/liter; KCl, 0.4 g/liter; glucose, 2 g/liter; NaH₂PO₄ · H₂O, 0.69 g/liter; Na₂HPO₄, 1.57 g/liter; pH 7.2 to 7.4) before addition to Caco-2 monolayers. After incubation for 1 h at 37°C, monolayers were washed three times with GKN solution at 37°C and then lysed with 1 N NaOH. Cell lysates were added to 10 ml of scintillation liquid (Microscint 20; Packard Bioscience Company) and counted in a RackBeta Spectral beta counter (LKB Wallac, Turku, Finland).

Proliferation assays. Since trophozoites are highly prone to divide, bacterial supernatants that inhibit proliferation may control the level of infectivity. We used different experimental conditions to examine this. In the first of these (protocol I), the bacterial supernatants were allowed to interact with the trophozoites for 1 h before the effect on proliferation was assessed. In the second

and third experiments (protocols II and III), trophozoites were coincubated with spent culture supernatant without pretreatment.

Protocol I. Fifty microliters of trophozoite suspension (2.2 \times 10⁵ parasites/ml) in TYI-S-33 medium was inoculated into 0.5 ml of bacterial supernatant or its control. After 1 h of incubation at 37°C, parasites were detached from the tubes by incubating at 0°C for 10 min, and 100 μ l of the suspension was added to individual wells of a 96-well tissue culture plate (Nunc). To each well was added 200 μ l of TYI-S-33 medium and 10 μ l of [³H]thymidine (21 Ci/mmol, 1 mCi/ml; Amersham Life Science). Plates were then incubated at 37°C in anaerobic jars (GasPack Plus) for 24, 48, 72, or 96 h. Trophozoites were detached from the plates by incubation at 0°C for 10 min, harvested (Harvester Filtermate 196; Packard Instrument Company), and the incorporation of [³H]thymidine was measured by using a microplate scintillation reader (Packard).

Protocol II. Two hundred microliters of trophozoite suspension (1.1 \times 10⁴ parasites/ml) was mixed with different volumes of either bacterial supernatant or its controls, with final dilutions ranging from 10 to 33%. Samples were incubated at 37°C for 24, 48, 72, or 96 h in 96-well tissue culture plates, the parasites were harvested, and the [³H]thymidine incorporation was evaluated as described above.

Protocol III. Trophozoites were grown in Dulbecco modified Eagle medium supplemented with 0.19% cysteine HCl, 0.95% casein hydrolysate, nonessential amino acids, 20% fetal calf serum, 12 IU of penicillin/ml, and 12 μ g of streptomycin/ml. Then, 1 ml of trophozoite suspension in the same medium was added to differentiated Caco-2 cells cultured in 24-well plates. Next, 0.5 ml of spent culture supernatant or control was added. Plates were incubated in a CO₂-air atmosphere for 48 h at 37°C. To evaluate parasite proliferation, trophozoites were dislodged at 0°C for 15 min, and counts were determined in a hemocytometer.

Control experiments were performed by adding lactic acid to MTYI medium to obtain the same pH as the spent culture supernatants. The pH of the controls was then adjusted to 6 or 7.

Scanning electron microscopy. Caco-2 cell monolayers incubated with trophozoites were fixed in 2.5% (vol/vol) glutaraldehyde for 16 h at 4°C. Postfixation was performed with osmium tetroxide at room temperature for 2 h. Smears were dehydrated in a graded series of ethanol solutions. Finally, samples were critical-point dried by using CO₂, gold coated, and examined by using a Philips SEM505 at an accelerating voltage of 30 kV.

Flow cytometric analysis. A total of 1.5 ml of TYI-S-33 medium containing 10⁵ trophozoites/ml was seeded into 24-well tissue culture plates, and 650 μ l of culture filtrate supernatant or their controls was added. The plates were then incubated at 37°C for 24 or 48 h, and the trophozoites were detached by cooling, washed with GKN, and permeabilized with 70% ethanol. After being washed, the samples were incubated at 37°C for 30 min with propidium iodide (Sigma) at 5 μ g/ml and RNase at 100 μ g/ml (Sigma). Flow cytometric analysis was performed by using a FACScan TM flow cytometer with blue-green excitation light (488 nm; Becton Dickinson, Basel, Switzerland).

Characterization of the anti-giardiasis factors. To estimate the molecular size of the exocellular factors produced by bacterial cultures, spent culture supernatants were exhaustively dialyzed against GKN solution at 4°C (molecular mass cutoff, 1,000 Da). To assess thermal stability, spent culture supernatants were heated at 90°C for 30 min. Dialyzed or heated supernatants were used in proliferation assays as above indicated.

Statistical analysis. The effect of the different treatments was evaluated by analysis of variance and paired *t* test. Analyses were performed with SYSTAT software (SYSTAT, Inc., Evanston, Ill.).

RESULTS

Since MRS is the most commonly used medium for the growth of lactobacilli (10), this medium was tested for its effect on *G. intestinalis*. When trophozoites were grown in TYI-S-33 medium containing 33% MRS broth, [³H]thymidine incorporation was 6% of that observed in TYI-S-33 medium alone. In a similar experiment with MTYI medium instead of MRS, the incorporation of radioactivity was 70% of that seen with the control (data not shown). On the basis of these findings, lactobacilli were grown in MTYI medium.

When trophozoites of strain WB were coincubated with differentiated Caco-2 cells, the parasites attached to the cell brush border via their ventral surface (Fig. 1A). Preincubation

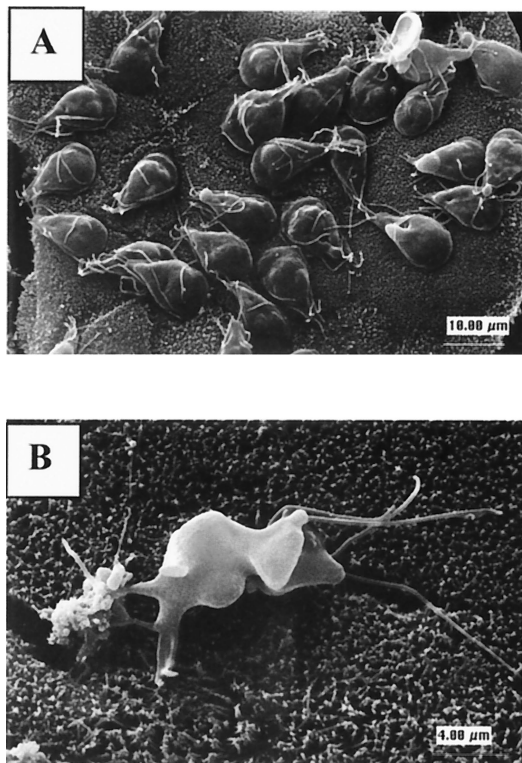


FIG. 1. (A) Adhesion of untreated trophozoites of *G. intestinalis* WB on differentiated Caco-2 cells. (B) Trophozoites preincubated with La1 culture filtrate supernatant prior to adhesion assay.

of trophozoites with neutralized spent supernatants of La1 at either pH 6 or pH 7 did not significantly influence parasite adhesion to the epithelial cell surface compared to control treatments (data not shown). Interestingly, scanning electron microscopy revealed that the trophozoites which were treated with La1 spent culture supernatants and which were still attached to Caco-2 cell monolayers had a morphology indicative of cellular damage (Fig. 1B). Taking into account that damaged trophozoites might be unable to grow, we studied the growth kinetics of trophozoites after different treatments.

Figure 2 shows incorporation of [³H]thymidine by the WB strain. When coincubated with 33% MTYI medium in TYI-S-33, counts already differed from the background levels after 24 h ($P < 0.01$). Proliferation of the trophozoites continued to confluence at 72 to 96 h. Preincubation of trophozoites with MTYI alone, prior to incubation with TYI-S-33 containing 33% MTYI, resulted in a slightly slower growth curve, showing significant differences between treatments at 24 h ($P < 0.05$) and 48 h ($P < 0.01$).

Preincubation of *Giardia* trophozoites with spent culture supernatants of different LAB strains showed that significant inhibition of proliferation was only attained with two of eight strains tested (data not shown): La1 and *L. acidophilus* NCC2628. Subsequent experiments were performed with the probiotic strain La1. Preincubation of *Giardia* trophozoites with La1 spent culture supernatants, followed by incubation of the suspension in TYI medium (protocol I), led to a strong inhibition of [³H]thymidine incorporation after 72 and 96 h (Fig. 3). The effect of spent culture supernatants was significantly

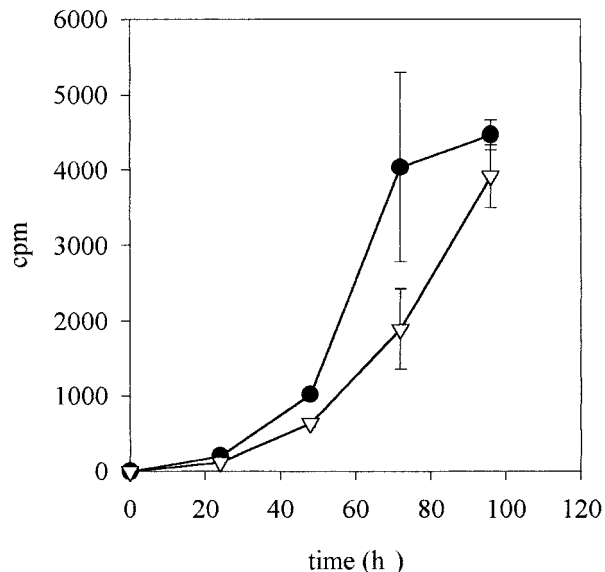


FIG. 2. Kinetics of thymidine incorporation for trophozoites of *G. intestinalis* WB. Symbols: ●, trophozoites were incubated in 66% TYI-S-33 plus 33% MTYI; ▽, after incubation in MTYI medium for 1 h at 37°C, trophozoites were incubated in 66% TYI-S-33 plus 33% MTYI. The error bars are not shown when they are smaller than the symbols.

different from the controls at the same pH ($P < 0.001$).

The inhibition of [³H]thymidine incorporation in trophozoites coincubated with 33% (vol/vol) of La1 spent culture supernatant is depicted in Table 1. For trophozoites exposed to

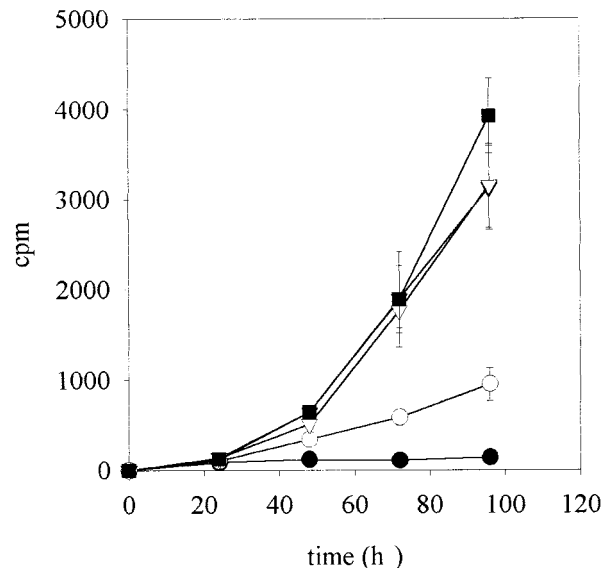


FIG. 3. Kinetics of thymidine incorporation for trophozoites of *G. intestinalis* WB preincubated with La1 spent culture supernatants at pH 6 (●), pH 7 (○), control pH 6 (▽), control pH 7 (■), and MTYI (▼). The effects of La1 spent culture supernatant at pH 6 and 7 were significantly different from the controls ($P < 0.001$). The effects of La1 spent culture supernatant at pH 6 and 7 were significantly different ($P < 0.05$). Values represent averages from three determinations \pm the standard deviation. The error bars are not shown when they are smaller than the symbols.

TABLE 1. Kinetics of inhibition of [3 H]thymidine incorporation by *G. intestinalis* WB grown with 33% (vol/vol) La1 culture filtrate supernatants

Time (h)	Mean % inhibition \pm SD ^a at:	
	pH 6	pH 7
48	94 \pm 0.8	18 \pm 5
72	96 \pm 0.9	27 \pm 5
96	98 \pm 0.1	53 \pm 1

^a The results are means of three determinations \pm the standard deviation. The percent inhibition was calculated as the counts per minute (cpm) of the treatment group/cpm of the control group \times 100.

supernatants at pH 6, the incorporation was inhibited by approximately 96% compared to those exposed to control treatments and was not time dependent ($P > 0.1$). In contrast, when supernatants at pH 7 were used, the percentage of inhibition increased from 18 to 53% after 48 and 96 h, respectively.

Coincubation of trophozoites with increasing concentrations of La1 spent culture supernatants leads to an exponential decrease in [3 H]thymidine incorporation (Fig. 4). Significant differences were found when supernatants at pH 6 and 7 were compared ($P < 0.005$). Supernatant concentrations as low as 10% produced two and three times lower incorporation of thymidine at pH 7 and 6, respectively, compared to the MTYI medium control. Controls performed with MTYI medium acidified with lactic acid and neutralized to pH 7 also produced a dose-response inhibition of the *Giardia* proliferation. However, thymidine incorporation was significantly higher ($P < 0.01$) compared to samples coincubated with spent culture supernatants at either pH 6 or 7. Antiproliferative activity was also observed in the presence of Caco-2 cells (data not shown).

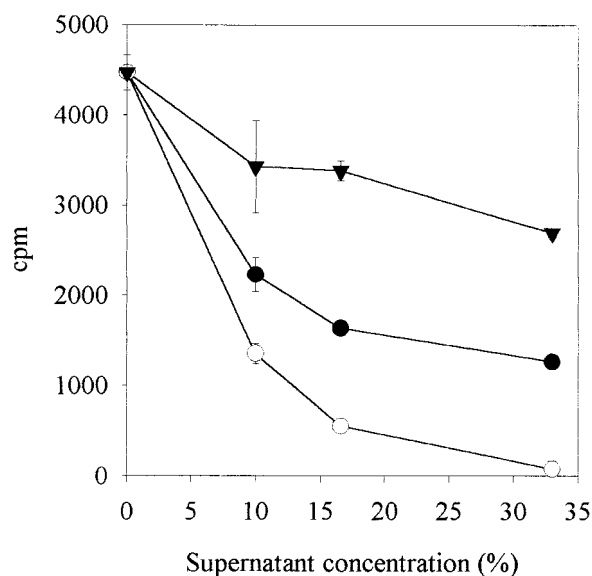


FIG. 4. Thymidine incorporation by trophozoites of strain WB after incubation for 96 h with concentrations of La1 culture filtrate supernatants ranging from 33 to 10%. Symbols: \circ , spent culture supernatant at pH 6; \bullet , spent culture supernatant at pH 7; \blacktriangledown , control (pH 7). Values represent averages from three determinations. The error bars are not shown when they are smaller than the symbols.

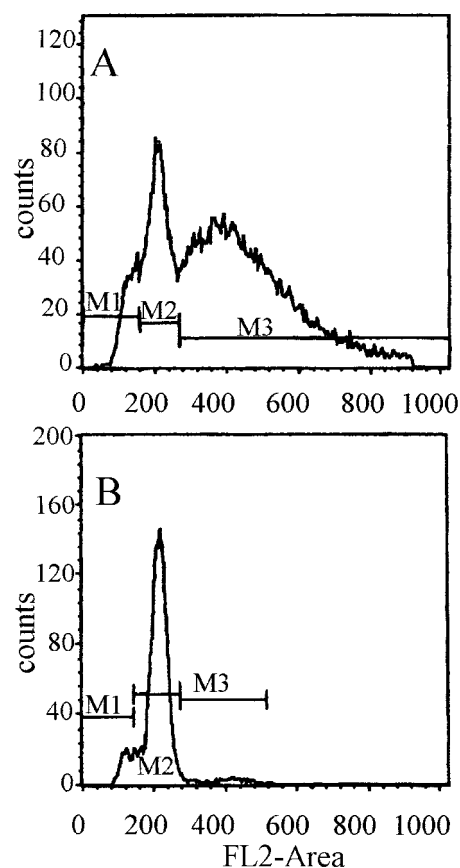


FIG. 5. Flow cytometric analysis of the DNA content of trophozoites of *G. intestinalis* WB grown for 48 h in TYI-S-33 medium (A) and in TYI-S-33 medium with 33% of La1 spent culture supernatant at pH 6 (B). Regions represent the sub- G_1 peak (M1), the G_1 phase (M2), and the G_2/M phase (M3).

Flow cytometric analysis of the trophozoite's cell cycle is shown in Fig. 5. When trophozoites were incubated for 24 h in TYI-S-33 medium, three well-defined regions were observed. Region M1 (sub- G_1 peak) represents apoptotic cells, region M2 represents cells in G_1 phase, and region M3 represents cells in G_2/M phase (cells in the Gap 2 period and mitotic cells). While 68% of actively dividing trophozoites were in G_2/M phase (Fig. 5A), only 4% of the trophozoites were in this phase 48 h after incubation with 33% of spent culture supernatant at pH 6 (Fig. 5B). Identical figures were obtained with spent culture supernatants at pH 7 (data not shown).

To improve our knowledge of the inhibitory product(s) present in the spent culture supernatants, the supernatants were dialyzed against GKN by using a molecular mass cutoff membrane of 1,000 Da. After exhaustive dialysis the inhibitory activity of the spent culture supernatant was drastically reduced compared to the neutralized nondialyzed one (Fig. 6).

Further characterization of the inhibitory activity was performed by examining the sensitivity of the responsible factor(s) to heat treatment. Supernatants were heated at 90°C for 30 min, and the inhibitory activity was significantly abrogated (data not shown).

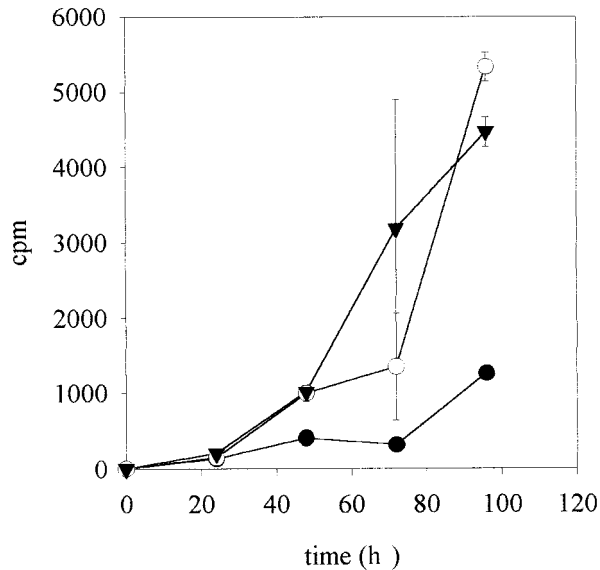


FIG. 6. Kinetics of thymidine incorporation by trophozoites of *G. intestinalis* WB incubated with 33% dialyzed spent culture supernatant (○) or nondialyzed spent culture supernatants of strain La1 (●) and MTYI (▼). The error bars are not shown when they are smaller than the symbols. Dialyzed versus nondialyzed spent culture supernatant differed in their inhibitory activities at 48 and 96 h ($P < 0.05$). At 72 h the difference was at the $P = 0.06$ level. Nondialyzed spent culture supernatant was more inhibitory than MTYI only at 96 h ($P = 0.006$).

DISCUSSION

It has previously been shown that the composition of the intestinal microflora may affect the colonization of the mouse gut by giardia trophozoites (29). Since oral administration of probiotic LAB is thought to modify the composition of the intestinal flora and thereby provide protection against bacterial pathogens (7), the purpose of the present study was to determine whether such bacteria could offer similar protection against giardia. To this end, we studied the effect on *G. intestinalis* of different lactobacilli, including the probiotic bacteria, *L. johnsonii* La1, "in vitro."

Preliminary experiments showed that the preincubation of Caco-2 cells with suspensions of *L. johnsonii* La1 did not inhibit the complex adhesion of trophozoites to cell monolayers (data not shown). This interaction involves the ventral disk, several contractile elements, and hydrodynamic and mechanical forces, as well as lectin-binding factors (18, 21, 24, 25). The possibility remained that if an antagonistic effect of the bacteria against the parasite existed, it could result from the release of bacterial metabolites or products more than a competitive exclusion or steric hindrance (3).

Because MRS medium produced a deleterious effect on giardia trophozoites, we had to formulate a new culture medium for lactobacilli. This medium (MTYI) is a modification of the TYI-S-33 medium and supported the growth of lactobacilli without any damage to trophozoites. When TYI-S-33 medium was supplemented with MTYI, good growth of trophozoites was achieved. The absence or lower concentration of bovine serum in the MTYI and the TYI-S-33 supplemented with MTYI, respectively, could explain the slightly lower growth of

trophozoites in these media compared to TYI-S-33 medium alone.

Although La1 spent culture supernatants failed to inhibit trophozoite adhesion to enterocyte-like cells, dramatic changes of the morphology of attached parasites were observed. This cytopathic effect correlated with an inhibition of the trophozoite proliferation which does not seem to be only due to the presence of lactic acid. During fermentation, La1 produces ca. 200 mM lactic acid (L and D isomers). It also produces, in very minor quantities, malic and propionic acids. Controls were performed by adding lactic acid to the culture medium to obtain a final pH similar to that of the supernatants that, in fact, was very close to the 200 mM level produced during fermentation. After being neutralized to pH 6 or 7, these controls showed a very low antiproliferative capacity.

Since trophozoites treated with La1 supernatant were able to attach to Caco-2 monolayers and displayed abnormal morphology (Fig. 1B), adherence of parasites to enterocyte-like cells was not considered an appropriate method for further examination of anti-giardia effects of the La1 supernatant. However, since infection requires both attachment of trophozoites to epithelial cells and subsequent proliferation of the parasites, we examined the effect of La1 metabolites on the proliferative capacity of the trophozoites.

Trophozoites which were pretreated with the spent culture supernatants of the La1 (pH 7), before addition of [3 H]thymidine, exhibited an impaired proliferative response. When culture supernatants at pH 6 were used, trophozoite proliferation was completely abolished (Fig. 3). It should be noted that the concentrations of the antagonistic substances in the preincubation step are very high and that a more realistic approach should take into account that the intestinal secretions will dilute the bacterial exocellular factors. However, by using the second experimental procedure without pretreatment of the trophozoites, the addition of spent culture supernatants in concentrations as low as 10% (vol/vol) caused a significant decrease in trophozoite proliferation after 96 h of incubation (Fig. 4). These findings suggest that in a stricter experimental system, with low concentrations of the inhibitory substance(s), an anti-giardia activity is still observed. Although the effect was strongly pH dependent and a contribution of lactic acid to the activity is certainly possible, other substances seem to be involved.

According to previously reported results (6, 14), parasite growth increases when trophozoites are coincubated with epithelial cells. However, the antiproliferative capability of spent culture supernatants of La1 strain was observed even in the presence of differentiated Caco-2 cells (data not shown). In our experimental system, stimulation of trophozoite growth by enterocyte-like cells is not high enough to overcome the antiproliferative activity of the spent culture supernatants.

Coincubation of trophozoites with La1 supernatants caused cell cycle arrest in the G_1 phase (Fig. 5), with no DNA replication detected even after 48 h in culture. These findings contrast with those previously described for lectins, which led to arrest in a later phase (G_2/M) of the cell cycle (27). Interestingly, differentiation into cysts takes place from the G_2 phase (2), and thus the extracellular factors produced by La1, can prevent giardia from producing cysts that in turn will diminish the spreading of the parasite.

Flow cytometric data and proliferation assays suggest that cells are arrested in their cell cycle rather than programmed for apoptosis or killed by the activity of the spent culture supernatant. The loss of the inhibitory activity after dialysis supports the contention that a low-molecular-mass substance is involved in this activity. In addition, the activity is also lost after heat treatment. The interpretation of these data is not simple, and further studies are required to identify the responsible factor(s). The precise mechanisms and products responsible for the inhibition in parasite cell division thus remain unknown and merit further research.

The antagonistic effect of *L. johnsonii* La1 on *G. intestinalis* suggests that modulation of the microbial ecology in the intestine, through the administration of probiotics, may constitute an interesting approach for the prevention of giardiasis. Although the underlying mechanisms of such antagonism are unknown, decreasing trophozoite proliferation may be a relevant target to clear parasites from the host or to reduce pathogenic effects. For the moment, the inhibitory effect can be ascribed either to a common product of bacterial metabolism or to a combination of products rather than to a specific antimicrobial compound. Nevertheless, the fact that La1 has GRAS (generally regarded as safe) status and is able to modify the parasite's cell cycle encourage further research on this subject.

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