Study the Effect of Human –Derived Probiotic Bacteria on the Viability of Cryptosporidium Parvum

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Abstract
This study was designed to evaluate the ability of Lactobacillus acidophilus probiotic bacteria to infect Cryptosporidium parvum viability by different bacterial dilutions and status. Cryptosporidium from stool samples of diarrheal patients were collected and diagnosed by parasitological methods, then molecular methods and cultivation in cell lines were done (Human adenocarcinoma cell). Probiotic bacterial dilutions (1:1, 1:2 and 1:3) and status (supernatant, pellet and dead bacteria) in De Man Rogosa Sharpe (MRS) mediu, the treatment of Cryptosporidium Parvum by adding Lactobacillus acidophilus dilutions and status were then incubated at incubation periods (24 hr, 48 hr, and 72 hr), detection of the Cryptosporidium count was carried out microscopically, and the parasite's viability by MTT and flow cytometry technique. The results showed that Lactobacillus acidophilus has a great ability to treat Cryptosporidium parvum by inhibiting the activity and decreasing the count with highly significant differences.
Keywords: Cryptosporidium, Lactobacillus acidophilus, probiotic bacteria, flow cytometry

1. Introductionn

Intestinal parasite infections (IPIs) have been identified as one of the world's significant public health issues. According to WHO estimates, over three billion individuals are infected with IPIs worldwide, yet only 450 million are symptomatic (1).
Cryptosporidiosis is one of the most frequent IPIs, and their method of infection is strongly dependent on the fecal oral rout (2). This method of infection can be acquired directly or indirectly through person-to person transmission or indirectly by the consumption of contaminated food or objects (3).The enteric parasite *Cryptosporidium Parvum* is one of the most widely distributed agents, causing gastrointestinal illnesses in humans and many other vertebrates, *Cryptosporidium* oocyst-contaminated food and water are the most Probiotics are live microorganisms that provide a health benefit to the host when given in sufficient concentrations, the use of probiotics in the treatment of parasitosis has been suggested due to their favorable benefits in animal models and people, such as a reduction in the time of gastrointestinal symptoms and parasite load, probiotics have been investigated as an alternate treatment for protozoa in the intestine. In this review, we looked at how effective probiotics are at treating and preventing these infections. *L. acidophilus* will be the focus of the present research (6).

2. Materials and methods

One hundred samples of human stool were examined by wet mount to investigate the presence of *Cryptosporidium parvum.* Non-bloody and bloody diarrhea stool samples were chosen at random for the study, stained by modified acid fast stain and the Polymerase Chain Reaction method for complete detection.

1- Concentration Method for *Cryptosporidium* purification (7)

Stool sample containing oocysts mixed in a sterile container with 10 times its volume of water, the resulting solution was passed through a 3-layer sterile gas ,filter and remove excess sample material, then centrifuged at 400 rpm for 10 min, the precipitate mixed again with 10 times water

important sources of infection for humans and animals, and the parasite can also be transmitted to humans through direct contact with infected animal,infection with *Cryptosporidium parvum* in birds can cause digestive problems, respiratory problems, and renal problems (4).
The use of drugs such as nitroimidazoles, nitrofurans, and phytotherapics is commonly highlighted among the forms of treatment for intestinal parasites and their symptoms; however, due to an increase in resistance to these compounds, there is an urgent call for the development of novel therapeutic strategies to fight the pathogen in a more healthy and effective way; therefore, nutritional interventions and modifications of the intestinal microenvironment through probing are commonly highlighted among the forms of treatment for intestinal parasites and their symptoms (5).

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and centrifuged at 400 rpm for 5 min, five ml water added to the resulting precipitate, the obtained solution was slowly added to 3 ml of 0.85 M sucrose, the resulting solution is centrifuged at 600 rpm for 10 min, the accumulated that containing parasite slowly transferred to another tube, the solution washed twice with water, antibiotics are added and kept in distilled water at 4 ° C for 7 days.

2- Perform PCR technique to confirm the microscopic method

DNA extraction (8)

After the QIAGEN DNA stool mini kit was used to extract DNA, 120-180 l of feces diluted with normal saline was poured into a 2 ml centrifuge tube and 1.4 ml of ASL buffer was added. After the vortex, the tube was placed in boiling water for 5 minutes and centrifuged at 14000 rpm for one minute. After one minute, the supernatant was transferred to a tube and added a tablet from the kit called Inhibit and Vertex to dissolve the tablet. After one minute, the supernatant was centrifuged at room temperature at 14,000 rpm for three minutes and then transferred to 1.5 ml and centrifuged at 14,000 rpm for three minutes. At the same time, 15 l of proteinase k was added to a 1.5 ml tube, 200 l of step 3 liquid was added to the tube containing proteinase k, and 200 l of absolute ethanol was added to the solution and mixed, then centrifuged slowly to collect all the solution at the bottom of the tube. The Ciamp column was then placed in a new 2 ml tube and 500 l of 1 AW buffer solution was added, and centrifAdd two AW buffers to it and centrifuge at 14,000 rpm for three minutes, then take the Kiamp column into a 1.5 ml tube, add 200 microliters of AE buffer, leave the tube at room temperature for one minute, then centrifuge for 14 minutes at 14,000 rpm to remove the DNA from the column, I discarded the DNA solution in the refrigerator for short-term use and in the freezer at -20 for a long time.

A nanodrop device was used to measure the extracted DNA, and two pairs of 2001 Xiao primers were used to amplify S-rRNA fragment. Primary primers amplified the gene's kb fragment, and secondary primers amplified the gene's 864–826 bp fragment.

DNA extraction procedure (9)

Approximately 300 l of fecal suspension was washed three times with distilled water to remove traces of dichoromate, and then genomic DNA was extracted using the DNAzol kit according to the manufacturer's instructions, with the addition of three freeze-thaw cycles (10 minutes). After resuspending the DNA samples in lysis (to rupture *Cryptosporidium* oocysts), the oocysts were frozen in liquid nitrogen. Thawing was carried out at 90° C in a water bath.

PCR Steps	Temp.	Time	Repeat
Initial Denaturation	95 °C	5min	1
Denaturatio	95 °C	30sec	30cycles
Annealing	55 °C	55sec	
Extension	72 °C	30sec	
Final extension	72 °C	5min	1

Preparation of Primers (10)

The A primers used in this study were prepared according to the manufacturer's recommendation by dissolving the lyophilized primers in an appropriate volume of nuclease-free water to yield 100 pmol/l as a stock solution. A working solution was prepared with a final concentration of 10 pmol/l by dilution methods.

PCR Product analysis

PCR products were analyzed by agarose gel electrophoresis. 1 gm. of agarose powder was dissolved in 100 ml of 1X TBE, then boiled in a water bath at 100 oC for 15 min, and left to cool at 55-60 oC. The comb was gently removed from the tray and 5 l of DNA sample were added in to each comb well. A 100 bp DNA size marker was loaded along with experimental samples. The gel tray was fixed in the electrophoresis chamber and filled with 1XTBE buffer.

Measurement the purity of DNA (11)

Nanodrop was used to measure the extracted DNA purity by measuring the absorbance at (260/280 nm) and quantifying the DNA concentration (ng/ml) (Adams and Otárola-Castillo, 2013).

Cell line(12)

Human adenocoarcinoma cells (HCT-8) obtained from the ATCC (CCL 244) were maintained in 25 cm2 of tissue culture flasks with biweekly splits, cells were grown to confluency in RPMI 1640 medium supplemented with 4.5mm glucose, 1.5mm sodium bicarbonate (Sigma), 100mm sodium pyruvate, 1 m HEPES buffer, 1% l-glutamine and 10% fetal bovine serum, 5% CO2 atmosphere at 37c, supernatant and MRS broth toxicity to HCT-8 cells was predetermined using Cell Titer 96t AQueous Nonradioactive cell proliferation assay kit, 96-well plate received 100 ml suspension of 104 HCT-8 cells in supplemented RPMI 1640.

Procedure of Cultivation (13)

Oocyst and sporozoite preparation of the parasite inoculation onto cell cultures, the process of establishing cryptosporidial infections in cell cultures with cryptosporidial oocysts and purified sporozoites, in vitro culture is established by inoculating whole oocysts, mixtures of intact oocysts, excysted oocysts, free sporozoites, purified sporozoites, approach can achieve

infection, but the efficiency of infection may be negatively impacted by oocyst age, excystation efficiency, or sporozoite integrity following purification, excystation will often proceed in the cell culture medium soon after inoculation onto host cells, even without specific treatment to trigger it, traditionally, oocysts have been suspended in a trypsin and sodium taurocholate solution (0.25% and 0.75%, respectively) prepared in a buffered salt solution (e.g., PBS or HBSS), simultaneously.

Unless the oocyst source is old (>6 months), I prefer to use 0.75% synthetic sodium taurocholate alone in DMEM basal medium, incubating at 37°C for 10 to 15 min (as a trigger) or for 45 to 60 min for total sporozoite release, the bleach washed out (or neutralized with 0.1% sodium thiosulfate), and the oocysts are inoculated onto host cells, treatment triggers excystation (or at least may enhance overall excystation rates) and can provide additional sanitization of the oocyst sample in order to prevent unwanted microbial contamination of the host cells., Most oocyst preparations processed and stored in the presence of 2.5% K2Cr2O7 rarely have viable microbial contaminants if aseptic techniques are used during the postpurification handling of the samples.

Preparation of Probiotic Bacteria

L. acidophilus preparation (14)

L. acidophilus (ATCC 4356) from AL-Kufa university cultured in MRS-Agar culture medium under microaerophilic conditions at 37 ° C for 18 hours, with the emergence of small or milky colonies and with full growth, 12% was cultured in nutrient medium with glycerol 5% for storage at -70 °c. confirmatory and diagnostic tests were carried out such as warm gammosis (for long bacilli, gram-positive, without spores), Albert staining (for bacilli with grains beside catalase test for complete diagnosis.

Treatment (15)

Treatment of *Cryptosporidium* done by probiotic bacteria (*L. acidophilus)* in dilutions (1:1, 1:2 and 1:5), duplicate RPMI control wells received an additional 100 ml of supplemented RPMI 1640. Duplicate test wells received 100 ml of diluted (1:1, 1:2, 1:5 in supplemented RPMI 1640). Supernatant, pellet and dead cell (statuses of bacteria) in MRS broth, plates were incubated for 24 hr 5% CO_2 atmosphere at 37C and viability of parasite determined, efficacy of bacterial MRS-Agar culture medium, topical solution containing *L. acidophilus* bacteria in three dilutions 1:1,1:2 and 1:5 on human adenocarcinoma cells, this study was performed as a multi plate. 100 µl of culture medium containing (10^5 × 1) cells was added to wells of plate and then 100 µl of bacterial MRS culture medium.

Enumeration of oocysts (16)

Cryptosporidium oocysts were enumerated before and after the treatment in three times 24 hr, 48 hr and 72 hr. Used fluorescent labeling and direct counting as described in (EPA Method 1622) which requires filtration, immunomagnetic separation of the oocysts from the material captured, then Enumeration of the target organisms based on the results of immunofluorescence assay, 4',6-diamidino-2-phenylindole (DAPI) staining results, and differential interference contrast microscopy, the purification steps of this method were not necessary because relatively clean DI water was used to prepare the oocyst suspensions, briefly, water samples were vacuum filtered onto a 0.22 mm pore size membrane filter (Fisher Scientific), and then 330 mL of immunofluoresence antibody solution (Waterborne Inc.) was applied to the filter. The fluorescently labeled oocysts were counted by epifluorescence microscopy at 400x with a Nikon microscope (model Eclipse E600) equipped with mercury vapor lamp, digital camera, and image analysis system, oocyst recoveries (calculated by comparison to hemacytometer counts of the oocyst stock solution) obtained using this simplified oocyst enumeration method typically ranged from 79% to 94%

MTT Protocol for Viability of *Cryptosporidium* (17)

Plates were incubated one additional hour at 37C, 5% CO2, and absorbance recorded at 290nm, the percent availability of parasite was calculated as [(mean absorbance of test wells–mean absorbance of RPMI control wells) /mean absorbance of RPMI control wells]*100, test treatments in wells with >85% viable cells were considered non-viable. Consequently, the highest concentration of each test and control at was deemed non-toxic to the HCT-8 cell monolayers (which was a 1:4 dilution) was evaluated in the cell-culture immunofluorescent (CCIF) assay. The pH of each 1:4 diluted test supernatant was 5.4 and the pH of the 1:5 diluted MRS broths was 6.3.

Flow cytometric viability assay (18)

The viability of oocyst pools before and after being subjected to the test and control treatments were determined by flow cytometry, 5106 oocysts were labeled with a FITC-conjugated monoclonal antibody specific for Cryptosporidium oocyst wall then suspended in 1ml water, incubated 5 min with 5 ml propidium iodide (1 mg/ ml in PBS, pH 7.2), and evaluated on a Becton Dickinson FACS Caliber. Oocysts were gated on the FSCFL1 dot plot and their viability determined in dilutions of bacterial suspension (1:1 ,1:2 , and 1:5) on the FSCFL2 plot using FACSComp software (version 3, 1996, Becton Dickinson)

3. Results

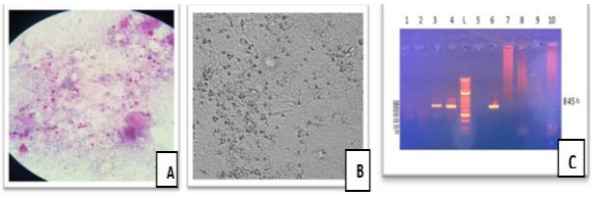

Figure (1) A/ Cryptosporidium by y modified ziehl-Neelsen Stain(100x)

One hundred sample from patients suffered from diarrhea for *Cryptosporidium* detection, beside 20 samples for healthy control, the results showed , positive samples were (35 %).

B/ C.Parvum cultivation in cell line (HT-29)
C/PCR image of cases were detected by conventional PCR product analysis for Cryptosporidium gene M (Marker ladder 845 bp).

Table (1) Represent the effect of using *L. acidophillus* dilutions (1:1, 1:2, 1:5) on the *C.Parvum* count by microscopic method.		
Control &dilution	Cell count (%) Mean+S.E	T- test (p-value)
Control	99.63±0.32*	P=0.001 High Sig *(P value ≤ 0.05)
1:1	11.01±0.29*	
1:2	15.57±0.35*	
1:5	30.34±1.80*	

Table (1) Illustrated the mean and standard error of using L. acidophilus dilutions in a treatment of Cryptosporidium and change the count microscopically before and aftertretment , the results showed L. acidophilusin in dilution of (1:1, 1:2 and 1:5) can decreased the count of B. hominis ,high significant differences documented (Mean±S.E was 11.01±0.29, 15.57±0.35 and 30.34±1.80) respectively comparison with control (Mean±S.E=99.63±0.32) at p value ≤0.05.

Table (2) mean and standard error L. acidophilus supernatant and the count of Cryptosporidium using microscopic technique count.		
Supernatant bacteria	Cell count (Mean±S.E)	ANOVA test p-value
Control	15133±66.67*	P=0.001 Sig *(P value ≤ 0.05)
24 hr.	8766.7±145.30*	
48 hr.	2033.3±33.33*	
72 hr.	530±25.17*	

Table (2) The mean and standard error of cell supernatant of *L. acidophilus* treated *Cryptosporidium* with different times from incubation (24 hr, 48 hr and 72 hr), the parasite microscopically counted. The results shows the supernatant status of *L. acidophilus* can decreased the count of *Cryptosporidium* with high significant differences, 24hr (Mean±S.E=8766.7±145.30), 48 hr(Mean±S.E= 2033.3±33.33) and 72 hr (Mean±S.E=530±25.17) respectively in comparison with control group.

Table (3) the mean and standard error of pellet L. acidophilus on the viability of Cryptosporidium using microscopic technique count.		
pellet bacteria	Cell count (Mean±S.E)	ANOVA test p-value
Control	16033±33.33*	P=0.001 High Sig *(P value ≤ 0.05)
24 hr.	9933.3±33.33*	
48 hr.	2150±76.38*	
72 hr.	550±50.00*	

Pellet L. acidophilus in a treatment of Cryptosporidium with different incubation period (24 hr, 48 hr and 72 hr) and count effecting determined in table (3) ,showed the pellet L. acidophilus decreased the count of Cryptosporidium with high significant differences, in 24hr(Mean±S.E=9933.3±33.33), 48 hr(Mean±S.E= 2150±76.38) and in 72 hr (Mean±S.E=550±50.00) respectively, also with high significant value at p≤0.001 in comparison with control wells

Table (4) the mean and standard error of dead *L. acidophilus* and its effects on the count of *Cryptosporidium* compared with control by using microscopic technique count.		
Dead bacteria	Cell count (Mean±S.E)	ANOVA test p-value
Control	15400±100*	P=0.001 High Sig *(P value ≤ 0.05)
24 hr.	11067±66.67*	
48 hr.	8733.3±120.19*	
72 hr.	1100±57.74*	

Using a dead L. acidophilus in the treatment of Cryptosporidium and determination count effected (table 4) with declined the mean and standard error of with different times from incubation (24 hr, 48 hr and 72 hr). The results shows dead L. acidophilus could decrease the count of Cryptosporidium with high significant differences in comparison with control group p=0.001(Mean±S.E=15400±100), also high significant value with different incubation period, it was 24hr (Mean±S.E=11067±66.67), after 48 hr (Mean±S.E=8733.3±120.19) and after 72 hr Mean±S.E=1100±57.74).

Table (5) different times of incubation of L. acidophilus supernatant and its effects on the viability of Cryptosporidium by using of MTT technique		
Supernatant bacteria	Percentage (Mean±S.E)	ANOVA test p-value
Control	99.63±0.32*	P=0.001 High Sig *(P value ≤ 0.05)
24 hr.	53.28±1.28*	
48 hr.	34.25±0.69*	
72 hr.	21.64±0.59*	

The mean and standard error of using supernatant *L. acidophilus* in a treatment of *Cryptosporidium* with different times from incubation (24 hr, 48 hr and 72 hr) by using MTT technique that showed in table (5), the results illustrated the supernatant *L. acidophilus* can inhibited the activity of *Cryptosporidium* with high significant differences at P value ≤ 0.001 after 24 hr,48 hr and 72 hr (Mean±S.E=53.28±1.28, 34.25±0.69 and 21.64±0.59) respectively, also with high significant value as comparison with control in very high significant value also.

Table (6) different times of incubation of L. acidophilus pellet and its effects on the viability of Cryptosporidium by using of MTT technique

pellet bacteria	Percentage (Mean+S.E)	ANOVA test p-value
Control	99.63±0.32*	P=0.001 High Sig *(P value ≤ 0.05)
24 hr.	62.23±0.49*	
48 hr.	40.1±0.3*	
72 hr.	15.15±0.44*	

Pellet L. acidophilus in table (6) used in treatment Cryptosporidium and determination the activity by MTT technique, the mean and standard error (Mean±S.E=62.23±0.49, 40.1±0.3and 15.15±0.44) (24 hr, 48 hr and 72 hr) respectively, results showed the pellet status of L. acidophilus can inhibited the Cryptosporidium with high significant value at P value ≤ 0.001, comparison with control in (Mean±S.E=99.63±0.32).

Table (7) times of incubation of L. acidophilus dead and its effects on the viability of Cryptosporidium by using of MTT technique.

dead bacteria	Percentage (Mean+S.E)	ANOVA test p-value
Control	99.63±0.32*	P=0.05 Sig *(P value ≤ 0.05)
24 hr.	67.56±1.15*	
48 hr.	56.32±0.51*	
72 hr.	25.04±0.15	

Using a dead L. acidophilus in the treatment of Cryptosporidium and determination of its viability was showed in table (7) declined the mean and standard error of different times of incubation (24 hr, 48 hr and 72 hr). The results showed that, dead L. acidophilus could inhibited the Cryptosporidium with high significant differences in comparison with control group (Mean±S.E=99.63±0.32), also high significant value with different incubation period, 24hr (Mean±S.E=67.56±1.15), 48 hr (Mean±S.E=56.32±0.51) and 72 hr Mean±S.E=25.04±0.15).

Table (8) Comparison between MTT and Flowcytometry Technique in determination the viability of cryptosporidium after treatment by L. acidophilus in different dilutions after 24 hr from incubation

Control &dilution	Viability (%) by MTT	Viability (%) by Flow cytometry	P≤0.05
Control	99.63±0.32	99.95±0.05	0.5 NS
1:1	28.64±1.28	28.96±2.15	0.9 NS
1:2	32.61±2.23	34.72±1.26	0.54 NS
1:5	48.04±1.01	48.06±1.75	0.99 NS

Detection the viability of Cryptosporidium after treated by L.acidophillus in different dilutions compared the results of MTT technique demonstrated in table (8), the results showed , the mean and standard error in the dilutions of (1:1,1:2 and 1:5) showed non-significant differences (0.9, 0.54,0.99) respectively. And as a comparison with control group at P value ≤ 0.05

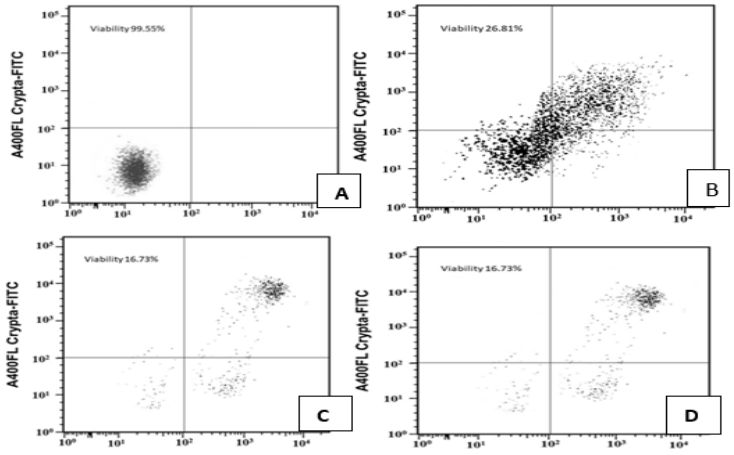

Figure (2) A: Flow cytometry dot plot propidium iodide stain showed control sample with viable cryptosporidium after 24 hr from incubation in the lower right quadrant (arrow).

A. B: Histogram showing a decreased cryptosporidium viability after treated by crude L.acidophillus (1:1 dilution, 24 hr) by flow cytometry (arrow)
B. C: Histogram showing more decreaced of the cryptosporidium viability after treated by crude L.acidophillus (1:2 dilution, 24 hr) by flow cytometry (arrow) compared with control.
C. D: Histogram showing (semi picture with 48hr) of the cryptosporidium viability after treated by crude L.acidophillus (1:5 dilution, 24 hr) by flow cytometry (arrow) compared with control.

4. Discussion

The antibacterial and antifungal activity of L. acidophilus has been well illustrated by researcher (19), the antiparasitic properties of L. acidophilus has been less studied (20). L. acidophilus in combination with other probiotics is beneficial in the prevention and treatment of Giardia lamblia infection in mice, Toxocara canis , Trichinella spiralis , and C.parvum (21).

Microscopic examination is the most common method in the assessment of stool specimens for Cryptosporidium, modified Kinyoun's acid-fast method is regarded as useful, successful excystation of viable sporozoites from Cryptosporidium oocysts is a critical step that is required for the initiation of a successful in vitro culture, several excystation protocols have been developed by different research groups for the achievement of high excystation rates (22). Results of excystaion studies varied between different research groups and several suggestions for the factors which affect this process have been postulated including reducing conditions, carbon dioxide, temperature, pancreatic enzymes and bile salts (5)

Del Coco, 2015 documented the mechanisms underlying the beneficial effect of the probiotic E. fecieum on C. parvum infection and could be attributed to several factors: competition for binding sites on the gut epithelium also declined about the antimicrobial effect of the peptide secreted by probiotic which may adversely affect the survival of microorganisms or changes in the microenvironment generated by the release of metabolic products of the probiotic, an important fact is that Cryptosporidium needs an alkaline environment to excyst, and the acidification of the medium induced by this lactic acid bacterium could affect this process reducing its viability(23). Lactobacillus caused marked improvement of intestinal epithelia-infected animal intestines, the intestinal integrity was completely restored after Lactobacilli treatment, probably through competing with the parasite for nutrient hindering or preventing the parasite colonization along the intestinal tract and improving the mucosal immunity to fight back the infection, these could explain both negative C. parvum antigen and CD3 overexpression at the local mucosal level in Lactobacillus treated group(23).

Cryptosporidium numbers reach to the minimum approximately 48 to 72 h after treatment with L. acidophilus, the used of MDCK cells for immunofluorescence in cover glass-bottomed culture chambers because the cells grow flat and the developing parasites are found in a narrow focal plane, facilitating image capture for subsequent software-assisted analysis, HCT-8 cells, however, grow much more three-dimensionally (hills and valleys), complicating image capture-based microscopic analyses (24)

The above results showed the effect of the dead L. acidophilus on count of Cryptosporidium, and this was in solidarity with another study conducted with the same idea in this aspect, agreement with Matthew et al., who dealt with Cell-free supernatants of L. acidophilus (LA) and L. reuteri (LR) cultures reduced the infectivity of bovine C. parvum and C. hominis in a cell-culture immunofluorescence (CCIF) assay by 21–42% and 30–35%, respectively(25). After 24hr incubation of C. parvum oocysts in the bacterial cell-free supernatants reduced oocyst viability 40–80% as determined by flow cytometric assay and oocyst infectivity was up to 95% determined by the CCIF assay, findings from this study concur with that of Foster et al. in that , incubation of oocysts in either MRS broth or Lactobacillus supernatant treatments reduces oocyst viability; in addition, the findings clearly showed that only the Lactobacillus supernatant treatments (and not the MRS broth) significantly reduce oocyst infectivity in cell culture, the reduction in bovine C. parvum and C. hominis viability and infectivity observed in this study in conjunction with previous reported reduction in bovine C. parvum viability (25)

Identifying specific mechanisms impacting pathogen virulence in response to probiotics consumption or diet may enable the development of targeted microbiota editing measures to mitigate the severity of cryptosporidiosis(26). Methods designed to detect metabolite modifications will be needed to supplement the information gained from 16S amplicon sequencing, lastly, enhancing the value of the rodent cryptosporidiosis model, the observed shift towards facultative anaerobes indicates common pathogenic changes in the human and rodent intestine in response to enteric infections(27).

In agreement with James et al., 2003 who studied the direct effect of the L. ruteri bacterial extract on the activity of the Cryptosporidium, and he was dealt with the viability of the oocyst test pool ,>95%, preliminary studies indicated that oocyst viability of >90% needed to ensure consistency and reproducibility of the assay, both bacterial cell free supernatants and their broth controls were effective in reducing oocyst viability (19–86% for Lactobacillus supernatants, vs 21–56% broth controls MRS, and 25–71% Bifidobacterium supernatants, vs 37–53% for RCM broth controls(28).

The present results showed that FC is potential to become a more precise method for the detected the viability of Cryptosporidium in cell line, additional

studies were underway to define the relationship between L.acidophillus effect on oocyst viability and L.acidophillus effect on infectivity in cell culture as recent reports suggest fluorogenic vital dye assays overestimate actual oocyst infectivity, particularly for aged oocysts, reduction of oocyst viability by the broth controls was not unexpected as such growth mediums contain proteolytic digests of proteins as a nitrogen base and residual enzymes could cause excystation of the oocysts (29).

In compatible with Karine, et al, that used flow cytometry, and demonstrated 1:100 MRS 1:5 and 1:10 RCM broth controls reduced viability over that of their respective bacterial cell supernatants unexpected and causes was unknown. It is plausible that certain compounds that were toxic to oocysts in the broth mediums are also utilized by the bacteria, thus, such compounds might be present in concentrations great enough to exert oocyst toxicity at the tested dilutions of the broths but are absent from the tested dilutions of the bacterial cell supernatants(30).

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