Study the Effect of Human –Derived Probiotic Bacteria on the Viability of *Blastocystis*

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Abstract

The purpose of this study was evaluation the quality of *Lactobacillus acidophilus* as probiotic in the treatment of *Blastocystis hominis* at various incubation periods, bacterial dilutions, and status. *Blastocystis hominis* was isolated from stool samples with diarrhea using parasitological diagnostic procedures, three bacterial dilutions (1:1, 1:2, and 1:3) and status (supernatant, pellet, and dead bacteria) were prepared in De Man Rogosa Sharpe broth, (MRS), then treated *Blastocystis hominis* rby adding *Lactobacillus acidophilus* dilutions and status and incubating it three periods (24 hr, 48 hr, and 72 hr), *Blastocystis hominis* counted.

Findings revealed that *Lactobacillus acidophilus* had the ability to treat *Blastocystistocystis*, by block the action and reduce the count with high significant differences, also the axenic cultures, showed *Blastocystis* inhibition by supernatant, pellet, and dead of *Lactobacillus acidophilus* from the 1st day of co-incubation, compared with control samples.

Key words: *Blastocystis*, *Lactobacillus acidophilus*, probiotic bacteria, (MRS) broth

1. Introduction

Intestinal parasite infections (IPIs) have been identified as one of the world's significant public health issues, with the World Health Organization (WHO) estimating that over three billion individuals are infected with IPIs globally [1]. Despite the fact that only 450 million are symptomatic, *Blastocystiasis hominis* is one of the most frequent IPIs, with a mechanism of infection that is heavily dependent on the fecal-oral pathway [2]. This method of infection can be acquired directly or indirectly through person-to-person transmission or indirectly through the consumption of contaminated food or items [3].

Blastocystis hominis is a eukaryotic protozoan parasite that belongs to the stramenopiles clade of heterokonts, whose members are commonly found in the intestinal tracts of humans and animals around the world, while many infections produce subclinical and asymptomatic disease in humans and animals, this parasite has been linked to diarrhea and irritable bowel syndrome [4]. Drugs such as nitroimidazoles, nitrofurans, and phytotherapics are routinely used; however, because to a growth in resistance to these chemicals, there is an urgent need for the development of novel therapeutic techniques to combat the pathogen in a more healthy and effective manner. As a result, dietary therapies and changes to the gut milieu via probiotic delivery could be a viable therapeutic option [5].

Probiotics are live microorganisms that provide a health benefit to the host when given in sufficient concentrations,it have been examined as an alternative treatment for intestinal protozoa because of their favorable benefits, such as a shortened period of gastrointestinal symptoms and parasite burden in animal models and humans, in this review, the looked at how effective probiotics are at treating and preventing these infections, *Lactobacillus acidophillus* was the subject of the current study [6].

2. 1.2Materials and methods

Strains of *L. Acidophillus* were supplied from Al_Kufa university, one hundred diarrheal stool samples were collected then examined by general stool examination as well as 20 normal stool samples as a control sample, the control group (sex and age) was matched with the patient study group comprised of 20 Iraqi healthy adults ranging in age from 18 to 55 years from Fatimat Alzahraa hospital.

1.3 Detection of Blastocystis hominis.

1.3.1 General Stool Examination

Macroscopic Examination

Examination of stool samples with the naked eye physio,logical characters included (color, consistency, odor, blood, mucus and the adult worms if present).

Microscopic Examination \ direct physiological normal saline smear.

Three slides were investigated for each sample, examined under 40 x power lens to detect the trophozoite, cyst and amoeboid of *B.* huminis [7].

1.3.2 Microscopic Examination of Concentrated sample

The concentration method used in this study was Modified Sheather`s solution,flotation technique, this technique for concentrating the cyst of intestinal protozoa and other parasitic stages for *B.hominis*, the principle of this technique based on the idea that any light weight of parasitic stages will float on the top [8].

Preparation of sample

In a centrifuge tube, 2 ml of fecal suspension were placed, washed with distilled water and centrifuged for two times, then three – quarters of Sheather `s solution was added to the tube,mixed with applicator wooden stick, then the tube was filled with Sheather`s solution to 1 or 2 cm from top, centrifuged at 500 rpm for 10 min [9].

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1.3.3 Wet Mount method

This examination was conducted utilizing the wet mounts method of feces sample performed by taking a small piece of stool with a wooden stick and placing it on a slide with a drop of normal saline, mixed with the stool sample well with the normal saline, carefully covering the slide to ensure the formation of air bubbles, and then examining under a microscope at low and high magnifications (10x and 40x), as well as using lugol's iodine solution in the wet mount, following the same procedure as the normal [9].

Staining with permanent stains

Ziehl-Neelsen Stain.

Modified ziehl neelsen-acid fast stain has been used in the current study for identification of *Blastocystis* cysts,a thin smear was prepared on a clear microscope glass slide using a little drop of the concentrated feces sample, after allowing the slides to air dry, they were fixed in 100% methanol for 3-5 minutes, fixed slides were placed on staining racks and saturated for 20-25 minutes with ZN carbol fuchsin before being rinsed under slow running tap water for de-colorization. 5% acid Alcohol was applied for 20-30 sec and the slides were rinsed under slow running tap water, the slides were then counter stained with methylene blue for 2-3 min before being rinsed under slow running tap water and left to air dry. After drying, the slides were viewed using a high power (100x oil immersion) lens with a drop of oil. The positive slides have a bright red backdrop and a blue background [10].

A- Staining of concentrated sample.

Modified Zehil Nelseen stain used to identify *Blastocystis hominis*. in stool sample, one drop of supernatant from centrifuged concentrated stool sample was taken, mixed with one drop of Meyer, s albumin on slide to make a smear. Fixed in 95% ethanol for 2-3 min. Air dried for 30 min, flooded the slide with carbol fuchsin, heated gently using alcoholic burner till little steam was begun, then let to stain for 5 min, rinsed the slide with gentle tap water, decolorized with 3% HCl for 30 sec, then rinsed with tap water,the slide was flooded with methylen blue for 1 min, then rinsed with tap water, air dried,and examined by microscope on 40x, and 100x [10].

B- Culture of *B. hominis*

A representative sample with four or more *B.hominis* parasites in the field of the slide was obtained and cultivated in modified Jones' medium, as described in item. Inoculated 50 mg of each fecal sample into an autoclaved screw-incubation cap tube with modified Jones medium, incubated the tubes for (24, 48 hr) at 37, after that, I inspected one drop of culture under a microscope for 24 and 48 hours and determined the number of cysts (C) or Trophozoites (T), determination can be done by the numbers of C or T in one ml of sample by using the equation:

No. of (C) or (T) X 15.25 (no. of drop in one ml) = no of (C) or (T) in one ml sample.

Subculture was done after (72 hr) by applying a small amount (about 1ml) of the old medium to the new media tube, and also incubated at (37°C) for (24 hr) [11].

1.4 Preparation of Probiotic Isolation

L. acidophilus

L. acidophilus (ATCC 4356) were supplied from AL-Kufa University and 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. It should be noted that at all stages, confirmatory and diagnostic tests such as warm gammosis (for long bacilli, gram-positive, without spores), albert staining (for bacilli with grains), catalase test were used in order to ensure that bacteria are lactobacilli [12].

1.4.1 Preparation the Status of bacterial suspension.

Lactobacillus acidophilus (ATCC 4356) from AL-Kufa university was inoculated in MRS broth and incubated for 18 hr at 37Cº in an aerobic jar, then the culture was centrifuged at (10000 rpm) for 10min. Supernatant and pellet status cells were prepared by filtered through (0. 22-Mm) pore size filters, while dead cells were prepared by exposure to UV light through 10-15 min, bacterial cells were taken, washed and suspended in to count (1.5×10^8 cell / 0.5ml) by MacFerland Standard [13].

1.4.2 Preparation of dilutions of bacterial suspension.

For preparation 1:1 dilution added 1 mL from bacterial growth to 1 ml DW, for a total volume of 2 mL and for preparation 1:2 dilution added 1 ml bacterial growth to 2 ml from DW. 1:5 dilution = 1/5 dilution = 1 part bacterial growth and 4 parts DW in a total of 5 parts [14].

1.5 Treatment

Efficacy of bacterial MRS broth culture medium, topical solution containing *L. acidophilus*, and performed as a duplicate plate (for each dilution and status for bacteria). 100 µl of culture medium containing nearly ($10^5 \times 1$) *B. hominis* cells were added to wells of plate then 100 µl of bacterial MRS broth medium. After prepared three dilutions 1:1, 1:2 and 1:5 from bacterial suspension was added to John's media that contained *B. hominis* in wells of plate. Also in other wells, 100 µl of supernatant, pallet and dead bacterial cells of *L. acidophilus* added to each well that contained the parasitic cell. In addition, control (wells with *B. hominis* cell but without added of *L.acidophillus*) was added to the two wells of the culture medium, the number of *B. hominis* cells was counted microscopically by using a heamocytometer chamber before and after treatment in three times 24 hr, 48hr and 72hr from incubation [15].

1.5.1 Enumeration of *B.hominis*

Parasites in solution were enumerated before and after the treatment in three days (24,48 and 72) microscopically as briefly. One ml of sample counted by added it to the neubauer slide with cover slide then examined under power 40x.

1.5.2 MTT protocol

Due to its positive charge and lipophilic nature, the MTT reagent can pass through the cell membrane as well as the mitochondrial inner membrane of live cells, and is converted to formazan by metabolically active cells,

mosmann created the MTT test in 1983 based on the chromogenic aspect of this redox chemical reaction, which allows for a colorimetric-based determination of intracellular formazan synthesis, preparation the work solution by mixed 100 µl from MTT (stock) to 400 µl from substrate, the treatment steps of *B. hominis* by dilutions and status of bacterial suspensions as above then incubated for 24 hr in 37°C / CO2 environment. 50 µl MTT working solution added per well to achieve a final concentration of 0.45 mg/ml. Incubated 1 to 4 hrs at 37°C. 100 µl solubilization solution also added to each well to dissolve formazan crystals. Mixed to ensure complete solubilization, recorded absorbance at 570 nm, the sufficiency of the used treatment (*L.acidophilus* probiotic bacteria), was measured by MTT protocol before and after the treatment by applying the equation that used by Ghasemi et al. [16], Maloney et al. [17].

3. Efficiency of treatment % =

Ratio of cell growth inhibition = average number of control cell –average number treated cell / average number of control cell **[18]**.

4. Results

From one hundred samples *B. hominis* detected, in 20 samples, the results showed the positive samples were (20%).the morphology of the parasite shwon in figure (1).

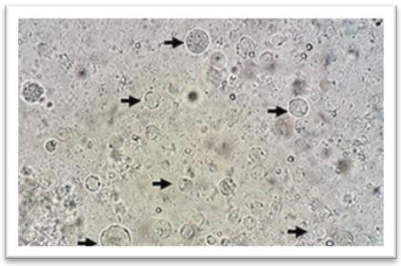

Figure (1)Microscopic examination for Blastocystis (granular, vacular and cyst shap)

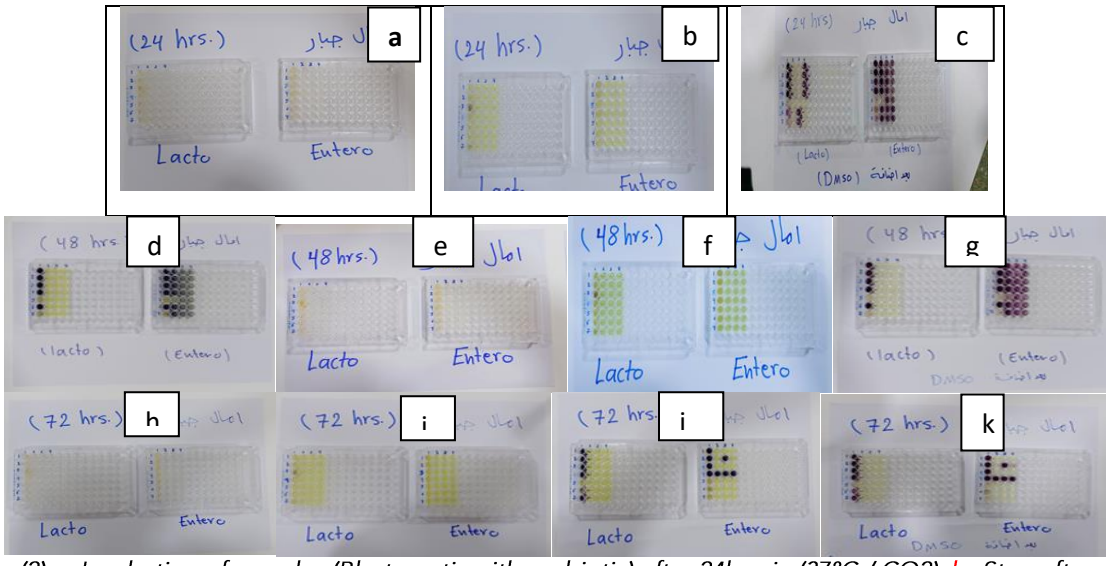

Figure (2) a- Incubation of samples (Blastocystis with probiotic) after 24hrs. in (37°C / CO2). b- Step after added working solution. c- Step after 4hrs adding working solution and (DMSO) in (37°C/CO2). D- Plate after 48 hrs in (37°C / CO2). E- Plate after add working solution. F- Plate after 4hrs adding working solution in (37°C/CO2).G- Plate after add DMSO Solution h-Plate after 72 hrs. in (37°C / CO2). i- Plate after add working solution. j- Plate after 4hrs adding working solution in (37°C/CO2). k- Plate after add ***DMSO Solution.***

Table (1) represent the effect of using *L. acidophillus* dilutions (1:1, 1:2, 1:5) on the *Blastocystis* count compared with control by microscopic.

Control &dilution	Cell count (%) Mean±S.E	T- test (p-value)
Control	99.33±0.33*	High Sig *(P value ≤ 0.05)
1:1	25±0.58*	
1:2	34±0.58*	
1:5	42±0.58*	

The mean and standard error of *L. acidophilus* dilutions in the treatment of *B.hominis* was demonstrated in Table 1. The results showed that all of the *L. acidophilus* dilutions (1:1,1:2, and 1:5) could decrease the count of *Blastocystis* with highly significant differences (MeanS.E = 250.58, 340.58, and 420.58), respectively, also observed highly significant differences as a comparison with control (untreated group) (MeanS.E = 99.330.33).

Table (2) Represent the effect of using supernatant *L. acidophillus* on the *Blastocystis* count by microscopic methd in different three incubations periods.

Supernatant of bacteria	Cell count (Mean±S.E)	ANOVA test p-value
Control	60666.67±666.67*	Sig at *(P value ≤ 0.05)
24 hr.	42666.67±1452.97*	
48 hr.	4766.67±145.3*	
72 hr.	130±15.28*	

Table (2) revealed the mean and standard error of the use of *L. acidophilus* supernatant in the treatment of *Blastocystis* with incubation periods of 24 hr, 48 hr, and 72 hr by microscopic count of the parasite. The results showed the supernatant of *L. acidophilus* causes decreasing in the count of *B. hominis* with very high significant value and p 0.001, and also showed high significant differences in three incubation periods (Mean S.E = 42666.671452.97, 4766.67145.3, and 13015.28) comparison with the control group with very high significant value also.

Table (3) Represent the effect of using pellet *L. acidophillus* on the count of *B.hominis* by microscopic technique count in different time of incubations		
pellet of bacteria	**Cell count (Mean±S.E)**	**ANOVA test p-value**
Control	69666.67±333.33*	**High Sig *(P value ≤ 0.05)**
24 hr.	42000±1527.53*	
48 hr.	3833.33±88.19*	
72 hr.	123.33±14.53*	

Table (3) illustrates the mean and standard error of using pellets of L. acidophilus in the treatment of *B. hominis* with different times from incubation (24 hr, 48 hr, and 72 hr). The results showed that the pellet status of *L. acidophilus* can decrease the count of *B. hominis* with a high significant value (p 0.001) after 24 hours of incubation (Mean S.E = 42000±152753, 48 hours (Mean± SE = 3833.33±88.19), and 72 hours (Mean S.E = 123.33±1453, throughout the daily incubation periods, the reason may be the concentrated status for the production of the inhibiting substances that are produced by the pellet bacteria, because the inhibition is very clear throughout the daily incubation periods.

Table (4) The mean and standard error of dead *L. acidophillus* on the count of *Blastocystis* by microscopic technique count in different time of incubations		
Dead bacteria	**Cell count (Mean±S.E)**	**ANOVA test p-value**
Control	68666.67±1855.92*	Sig *(P value ≤ 0.05)
24 hr.	52333.33±1452.97*	
48 hr.	2100±200	
72 hr.	876.67±14.53	

Table (4) disclosed the mean and standard error of dead *L. acidophilus* in the treatment of *B.hominis* after three incubation periods (24 hr, 48 hr, and 72 hr). The results showed a decrease in the count of *B.hominis* with high significant differences after 24 hr compared with the control group (52333.33±1452.97), while there was no significant value after 48 and 72 hr (2100±200 and 876.67±14.53), at p 0.05. That was possibly a result of the depletion of antiprotozoal substance that may be produced by dead *L. acidophillus* after more than 24 hr of incubation.

Table (5) Represent the effect of supernatant L. acidophilus on the viability of B. hominis compared with control in different time of incubations by MTT technique		
Supernatant bacteria	**Viability Percentage (Mean±S.E)**	**ANOVA test p-value**
Control	99.33±0.33*	High Sig *(P value ≤0.05)
24 hr.	67.37±0.32*	
48 hr.	46.57±0.47*	
72 hr.	23.13±0.88*	

By using MTT technique, the mean and standard error of using supernatant of *L. acidophilus* in the treatment of *Blastocystis* with different times of incubation (24 hr, 48 hr, and 72 hr) are shown in Table 5. The results showed the supernatant of *L. acidophilus* could inhibit the *Blastocystis* with a highly significant value after 24 hr, 48 hr, and 72 hr incubation (MeanS.E =67.37±0.32, 46.57±0.47, and 23.130.88), respectively, at p≤ 0.001, compared with the control group.

Table (6) Represent the effect of using pellet of *L. acidophilus* on the viability of *B. hominis* in different times of incubation by MTT technique.		
pellet bacteria	**Percentage (Mean±S.E)**	**ANOVA test p-value**
Control	99.33±0.33*	High Sig *(P value ≤ 0.05)
24 hr.	81.47±0.33*	
48 hr.	22.60±0.38*	
72 hr.	9.80±0.15*	

Table (6) explained the mean and standard error of using pellet *L. acidophilus* in the treatment of *Blastocystis* with different times from incubation (24 hr, 48 hr, and 72 hr). The results show the pellet status of *L. acidophilus* can inhibit *Blastocystis* with a highly significant difference (P value 0.001) and also a highly significant value with three incubation periods (Mean S.E =81.470.33), (Mean S.E =22.600.38), and (Mean S.E =9.800.15). Comparison with control (MeanS.E=99.330.33).

Table (7) Represent the effect of using dead *L. acidophilus* on the viability of *B. hominis* in different time of incubations by MTT technique.		
Dead bacteria	**Percentage (Mean±S.E)**	**ANOVA test p-value**
Control	99.33±0.33*	High Sig *(P value ≤ 0.05)
24 hr.	62.97±0.26*	
48 hr.	54.80±0.8*	
72 hr.	22.33±0.33*	

Table (7) explained the mean and standard error of using the dead status of *L. acidophilus* in the treatment of *B.hominis* with different times from incubation (24 hr, 48 hr, and 72 hr) by using the MTT technique. The results showed dead *L. acidophilus* inhibit *B.hominis* with significant differences at p≤ 0.001, with all three incubation periods of treatment (Mean S.E = 62.97 0.26), (Mean S.E = 54.80 0.8) and (Mean S.E = 22.33 0.33), respectively, compared with control (Mean S.E = 99.33 0.33).

5. Discussion

Intestinal microbiota have been shown in studies to play an important role in controlling intestinal diseases and keeping the intestines healthy, intestinal microbiota have been shown in studies to alter intestinal protozoa infection, the gut microbiota provides essential capacities for the fermentation of non-digestible substrates such as dietary fibers and endogenous intestinal mucus [19].This fermentation supports the growth of specialist microbes that produce short-chain fatty acids (SCFAs) and gases [20].

Furthermore, in-vitro cultivation and light microscopy were used as screening detection methods, every stool sample was cultured in Jones' medium to identify *Blastocystis hominis* cells [21]. Furthermore, considered in-vitro culture as the gold standard in detecting *Blastocystis hominis* cells and reported sensitivity. Stensvold et al., 2020 reported 100% sensitivity and specificity for culture when compared with formol-ethyl acetate concentration, trichrome staining,

and xenic in-vitro culture [18].

Roberts et al. observed 82.6% sensitivity and 100% specificity for cultur, factors like requirement for special equipment, high cost, and need for intensive labor limited its use in this study compared with PCR, the culture method is a cost-effective method for *B.hominis* detection in stool and it can yield valid prevalence estimates, in addition, the culture method also has a high detection rate since *B.hominis* is allowed to grow and propagate, even starting with a low infection [22].

A hemocytometer (Kova International) was used for multiple dilutions of the neat cultures, a drop plate method was utilized for the enumeration of bacterial colony-forming units (CFUs), to determine whether the presence of *L.acidophillus* affects *B.hominis* cell count in vitro, *Blastocystis* was individually co-incubated with representative *L.acidophillus,* reduced PBS condition used for co-incubation ensured a low oxygen environment necessary for *B. hominis* viability, while the simple PBS formulation minimizes potential exogenous growth factors that would otherwise complicate the assay, resulting in bacterial overgrowth [18].

Generally, *B. hominis* displayed decreased cell counts when co-incubated with *L. acidophillus*. Although previous studies have reported associations between *B. hominis* and gastrointestinal disorders, the protist's pathogenic potential and clinical significance still remains to be established [23]. To address the issue of *Blastocystis*' pathogenesis, it could be useful to determine whether *B. hominis* colonization is associated with gut dysbiosis, which is known to affect intestinal health [24].

Another agreement with Israa, *et al*, 2015 notification,gradually decreased in the numbers of the current study correspond with other research that deals with in vivo experiments and founds a reduction in the number of *B. hominis* cysts and trophozoites after treatment with lauric acid, this explained by its immune stimulatory effect through the ability to provoke lymphocyte proliferation and stimulation of T-cell proliferation via regulation of IL-2 production, a lymphokine necessary for T-cell proliferation, the effect of *lactobacillus* was evaluated on both vegetative forms and oocyst/gm stool, and the combination with metronidazole was used, these results in accordance with Amer *et al*. (2007). Who found that the combined therapy resulted in a reduction rate of 99.32 and 98.69% in both cysts and vegetative forms of *Giardia spp. Histopathological* examination of the infected control group revealed a profound effect on the structure of the intestinal mucosa, this effect was in the form of villous shortening and atrophy with infiltration of lamina propria with inflammatory cells. Additionally, *B. hominis* vegetative forms were detected in the intestinal lumen and in-between the villi [25].

Parasite in stool of mice since the first day of treatment *Blastocystis* disappeared in the day (12th) from treatment with Saccharomyces yeast, and when efficacy of treatment counted for both of *S. boulardii* founded that this yeast have a higher efficacy for treatment (87.45%).

Moyes *et al*. (2016) reported that *E. faecium* inhibited Eimeria after 24 hrs of co-incubation with a total bacterial CFU of 108 cells/mL. *Blastocystis* ST3 seems to be more resistant to its influence. In the Moyes experiment, the more effective concentration was 1.23 10^9 CFU/mL, as well as a longer incubation time of 4 or 5 days. Most likely, it lasts longer because *E. faecium* produces strong anti-microbial but no antiprotozoal compounds, and it does not compete for enteric adherence sites. More likely, this is caused by competing for nutrients and could be due to it being lactic acid bacteria, which colonizes differently and competes with *Blastocystis* differently.

Sherrington et al. [26] cleared up the question remains as to why the number of *B.hominis* cells decreased significantly after incubation with probiotics, one option may be the fact that the bacteria might be absorbed by *Blastocystis* at first while only low numbers of probiotic cells were in the incubated tubes, which supports protozoan proliferation, another option is that *E. coli* produces endotoxins, such as lipopolysaccharides (LPS), which could negatively influence *B. hominis* cells from inside after phagocytosis, which was observed in amoebic form, and destroy the parasite, which is in agreement with the present study [26].

Shaohua *et al.,* of the probiotic field can expect increased frustration with popular press writers. It's true that some dead microbes may have some health benefit, although evidence of such an effect is much lower than that available from controlled human trials on actual probiotics, as no studies have been conducted on this form of inactivated cells, but it's an interesting possibility, keeping probiotics alive in commercial products is a challenge. Research such as Prof. Cani's, 2020 targets an expanded range of microbes – many isolated from the human GI tract – that cannot be easily grown and stabilized in commercial products [27]. Further, these microbes lack the history of safe use that food-associated microbes have, and so administration of high numbers of these next-generation probiotics will require proof of safety. If these microbes can be killed and still deliver health benefits, the commercialization process could be simplified.

Current results showed the supernatant of *L. acidophilus* had the ability to inhibit the activity of the *B. homoinis,* which was detected by the MTT technique with a highly significant effect, and this may have come from the high ability of the bacterial extract to contain toxic and lethal substances*. There* was agreement with the other studies that are somewhat similar to the present issue, Sabaa et al. (2015) disclosed the potential of using *L. rhamnosus* and *L. lactis*, as well as *E. faecium,* as a prophylactic treatment against *B. hominis* colonization or as an additional treatment regimen in combination with standard drugs [28].

In a study that dealt with *E. histolytica* treated by *lactobacillus ssp*, mainly *salivarius,* were the dominant microorganisms isolated from the vagina of healthy women, they interfere with the colonization of pathogens by different mechanisms such as the production of organic acid, H2O2 and bacteriocins, *Lactobacillus* was found to be resistant to metronidazole, and Saccharomyces boulardii probiotics may have a beneficial

effect on *B. hominis* symptoms, according to Matsue et al. [29], also, when lauric acid was given alone or combined with metronidazole, experimental giardiasis with its anti-giarddial activity was better compared to the metronidazole drug [29].

Argenta *et al*. studied different concentrations of live *L. acidophilus* as well as cell free filtrate (CFF) derived from different concentrations of bacteria, the use of CFF is advantageous as a therapeutic in vivo because it can directly contact immune cells and its concentration is fixed, both live cells and CFF inhibited *Pseudomonas aeruginosa* biofilm formation, importantly, they also showed that high concentrations of CFF destroyed mature biofilm [30].

The present study disclosed the response of *B. hominis* in vitro when treated by different statuses of *lactobacillus*, the above results showed the activity of *L. acidophilus* in inhibiting *B. hominis* in successive incubation periods, the results were agreed with those of Nitya *et al.,* who dealt with the same idea but with live bacteria. Of the five probiotics chosen, individual treatments of *L. casei* and *E. faecium* showed a significant reduction of up to 71% in parasite survival only at higher CFUs, when the two probiotics were used in combination, the percentage of survival was reduced gradually further to 80% at a total CFU of 109 cells/ml of bacteria, the study lays the foundation for providing cost-effective prophylactic treatment for amoebiasis without the overuse of antibiotics [31].

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