

Original Research Article

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**The Effect of Different Concentrations of the Run and Stuck Bacteria
(*Lactobacillus fermentum*, *Lactobacillus delbrueckii*) in Trophozoite Growth
Entamoeba histolytica in Vitro**

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A B S T R A C T

This study describes the in vitro effect *Lactobacillus fermentum*, *L.delbrueckii* in *Entamoeba histolytica* was isolated from stools samples of patients with (amebic dysentery) and cultivated on Lock-egg medium to which sheep serum added. Than stud the effect of different concentrations (1.5×10^2 , 1.5×10^4 , 1.5×10^8) of stuck (*L.fermentum*, *L.delbrueckii*), and subject to examining after (24,28,72) hours of incubation, as well as to be observed the range of these concentrations' ability of the stuck and (*L.fermentum*, *L.delbrueckii*) to reduce and resist the amebic.*E. histolytica*, additionally to note the changes in formation that parasites suffering from, during the period of treatment, Comparing with metronidazole (flagyl).The results showed the effectiveness of bacteria stuck in the inhibition proliferation of parasite growth a higher compared degree with the (*L. fermentum*, *L.delbrueckii*) with the drug of metronidazole (Filagel) the treatment of effective concentrations reached to (1.5×10^2 , 1.5×10^4 , 1.5×10^8) of bacteria stuck (46.42, 88.65, 97.68)% respectively, after a 72h incubation. While the effectiveness of the treatment reached to (42.67, 31.57, 16.11) % respectively, after 48h incubation, while the (*L. fermentum*, *L.delbrueckii*) showed less efficient compared to the treatment of metronidazole, It was noted the decomposition of the plasma membrane and the small size of amoeba from the natural state and discoloration phases with bad dye trypan blue.

Keywords

Lactobacillus fermentum,
L.delbrueckii,
Entamoeba histolytica.

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Introduction

The amoebic dysentery are primitive unicellular microorganisms characterized with relatively simple life cycle, which is divided into two stages : the actively motile feeding stage (trophozoite), and the quiescent, resistant, infective stage (Cyst) (Murray *et al.*, 2013). The infection of

amoebic dysentery remains one of the most dangerous of the world's health problems and most serious intestinal diseases, especially in developing countries, so the speed and accuracy of screening for these parasites is considered the essential one in controlling the spread of mutants amoebic disease (Srivastava *et al.*, 2005). Infection of

ameba is spread all over the world and incidence is risen, as well, in tropical and subtropical areas, as well as with health and cultural low-lying areas (Pham *et al.*, 2011). About 90% of people with (amebiasis) the disease is developed with them form enteric (luminal form), so dysentery is happened and the invasion of the disease in less than 10% of cases, that can be fatal, statistics showed that the number of people with the parasite are about 50 million cases, hundred thousand of them are died per year (Bauman, 2015).

Probiotics are defined as useful and effective microscopic bodies that have an effective and key role in the natural protection from the harm of pathological biologist microorganisms, used in food support for the purpose of providing the beneficial effects to human health by restoring the natural balance of (Normal flora) and reduction of diseases, especially related to the digestive system (Al-Awad *et al.*, 2004). Thus, the treatment microscopic bodies are considered important defenses against many pathogens for many body parts, as they are characterized with the advantage by the impact of general mechanisms that determine vital boosters, which include the competition for binding sites on the intestinal surface, furthermore they participate in stimulating the immune system, and excretion of anti-materials and the prevention of pathogenicity, resist low (pH), and resist the acidity plus the ability to layer adhesion of epithelial lining of the intestines. (Forbes *et al.*, 2007). Lactic acid bacteria is considered, particularly Lactobacillus group of the important groups that are used in fermented food industry because of their health benefits and their ability to inhibit the growth of pathogenic bacteria to produce a lot of counter materials such as organic acids, hydrogen peroxide and bacteriocins. The components of this

group is characterized that their members are homogeneous in fermentation and they are grown at temperatures probably reach at 45°m, these bacteria are found in nature, in fermented foods and dairy products, as well as in the gastrointestinal tract, mouth and genital tract of the female human and animal (Reid *et al.*, 2001). It is worthy to say that the effect of the antagonistic between microbiology important has a key role in (Biological control), towards many of the parasites that infect on humans and reducing their spread. The studies on the impact of lactic acid on most parasites are rare, especially concerning the parasite *Entamoeba histolytica* as they are one of the most intestinal parasites that are common. That is why this study was to clarify the possibility of the use of lactic acid bacteria and their stuck for prevention and reduction of incidence with parasite amoebic dysentery, additionally to be used as a treatment for this parasite

Materials and Methods

Parasite collection and diagnosis of stool samples

Sixty stool samples were collected belonging to patients of different ages, in Al- Mahmudiya General Hospital, Children's Protection Hospital (Medical City) for the period from November 2014 to March 2015 putting in sterile plastic containers, full information on each patient recorded have been codified in card information (Questionnaire) included name, age, sex, residence, profession, test results come out, the type of drugs covered by the patient in the last week, Is the patient suffering from one of the following symptoms (fever - diarrhea - bloody stand - abdominal pain - weight loss). After confirmation of infection of the parasite through diagnosis of several tests, including

macroscopic examination where it is Note stool samples in terms of (consistency) and color and the presence of blood or mucus with feces, the microscopic examination as used the method of screening Wet mounts to conduct this examination, examining was done by taking a small sample of normal stool by using stick, by adding a drop of Normal Saline, placed on laced on a glass slide, and after a good mix put carefully the lid of the Caver slide in order to prevent the formation of air bubbles, then examined under a microscope under then they are Checked by taking a small part of a stool sample using a wooden stick by Minor powers (10X) and major powers (40X). For the purpose of viewing the presence or absence of each of the Trophozit and Cyst as well as Pus cell and red blood cells RBCs, it is also used several private diagnostic tools for ensuring diagnosis amoeba, equipped from (Cer Test Biotec). The Spanish Company so as to determine the Entamoeba.

Isolation of the parasite

The isolation of amoebas this case of (*Entamoeba histolytica*) of a stool sample by taking 1 gram of sample and mix it with 3 ml physiological saline, and passed through layers of sterile gauze for the purpose of removing the large slides of the emulsion before adding the sample after isolating amoeba stool added 0.5 ml of emulsion of the sample (media) to the center tube and then incubated tubes and put it in vertical condition in the incubator at a temperature of 37°C for 48 hours.

Preparation of Media

Locke-egg (LE) medium) was prepared, as it is one of the medias, type Xenic culture media culture media for the development of amoeba histolytica, which is (Diphasic

media) consists of two phases, is the first-phase solid phase which is down and poses (Slant), the second is the liquid phase, which is (Overlayer) the solid surface is leant. This media was prepared according to the method (Boeck *et al.*, 1925), which consists of two phases: Solid phase: hen's egg was taken, its upper part was fertilized with alcohol ethyl (70%) then the egg was broke in a graduated cylinder and mix the contents, taking 45 ml of the content of the egg and added to 12.5 ml of Lux solution and put the mixture in a blender to that smoothing and became emulsion. Then the emulsion was filtered through several layers of sterile gauze in the flask, funnel, distributed egg emulsion in the pipe laying 20 ml at 5 ml of the tube and the then a slope was made and at heights (1.5-3 cm). By placing them in boiling water bath for 15-20 minutes, after that the pipes were left to cool at room temperature.

Revitalization of bacteria (*Lactobacillus* spp)

Ready *Lactobacillus* spp is activated by suspending the 1 mg of the lyophilized bacteria in 5 mL of sterile warm water for three hours at a temperature of 37°C, then add stuck to a beaker containing 50 ml of Mann- Rogosa-Sharper (MRS) broth, and it is incubated under anaerobic conditions at a temperature of 37°C for 48 hours, then spread the stuck by sterile carrier with planned and arranged method to the center of MRS for individual places of bacteria, isolates were preserved in a refrigerator at a temperature of 4°C.

Preparation stuck lactic acid bacteria cells and filtrate

The developing colonies were transferred on MRS, the solid, into tubes containing 10 ml of broth MRS for the purpose of their revitalization, incubated anaerobically at a

temperature of 37°C for two (24-48) hour (6) and with concentration of (1.5×10^8) cells / ml by comparing the growth with a tube McFarland (0.5). Then conducted reduction for first half stuck, concentrations were prepared at $(1.5 \times 10^4, 1.5 \times 10^2)$ cells / ml then used to pollinate cultivated parasite.

Preparation of Stuck parasite

By using Hemocytometer for the purpose of preparing the stuck of trophozoite of *Entamoeba histolytica* with concentration of 0.1×10^6 trophozoite/ ml, were prepared of three replicates added 0.5 ml of bacterial Stuck to the tube containing the parasite growth incubated at a temperature 37°C heat, then results were recorded after (24-48-72) hours. Compared with the negative control (grown without the addition of the parasite stuck) and positive control (grown parasite added to a drug metronidazole).

Mortality rate

Growth rate of the parasite tested against propolis was calculated from the trophozoite count per ml, mortality rate of *E. histolytica* with respect to propolis at various concentrations was obtained as follow (Lwin *et al.*, 2004):

$$\text{Mortality rate (\%)} = \frac{\text{Count/ml (treated)}}{\text{Count/ml (untreated control)}} \times 100 - 100$$

Results and Discussion

Used *L.delbruekii* and *L.fermentum* bacteria returnee to the genus Lactobacillus, which were obtained a lyophilic them to test their of 37°C where the experiment effectiveness against the parasite inhibitory *E. histolytica*. After (24-28-72)h incubation at a temperature was performed with three replications for each concentration against parasitic isolation. The results showed that the percentage of viability trophozoite

phase for the condition of the weave when using bacterial cells stuck were directly proportional with increasing concentrations of the bacteria cells stuck *L.delbruekii* and *L.fermentum*, The bacteria cells leaky showed less efficient and as shown in (Table 1). While the results showed that the highest percentage of the effectiveness of inhibitory was stuck to a concentration of bacteria cells (1.5×10^8) cells / ml and by (97.68%) after 72h incubation, while it was lowest percentage of the effectiveness of inhibitory concentration was when (1.5×10^2) cells / ml and by (9.21%) after 24h incubation as shown in figure (1). The effectiveness inhibitory to a leaky bacteria cells showed less than stuck efficiency with the highest act of inhibitory his results when you focusing (1.5×10^8) cells / ml as a percentage of (35.95%) after 72h incubation and doing less inhibitory at concentration (1.5×10^2) cells / ml and a percentage of (1.31%) after 24h, and as shown in figure (2). Compared with the negative control and positive control (drug metronidazole), the treatment results also showed stuck of *L.delbruekii* and *L.fermentum* occur morphological changes of the trophozoites of *E. histolytica* was the emergence of cases the decomposition of the plasma membrane and pigmentation abiotic phases when using (dye trypan blue) figure (3) after 72h incubation. Statistical analysis showed that there were significant differences in for the value of LSD at the level ($P < 0.05$) between the concentrations of stuck *L.delbruekii* and *L.fermentum* in the number of trophozoites of *E. histolytica* case as well as the presence of a significant difference to the value of LSD at the level of ($P < 0.05$) what between the lap times, while no significant differences occur between the lap times of the transaction when the concentration (1.5×10^2) cells / ml as well as for the treatment of drug metronidazole.

Table.1 The percentages of viability for *E. histolytica* trophozoites LEM after treatment with stuck and leaky *L.fermentum* and *L.delbruekii* at different concentrations and different times

The percentages of viability for <i>E. histolytica</i> trophozoites(%)							
Time		24h		48h		72h	
		stuck	leaky	stuck	leaky	stuck	leaky
	1.5×10^2	90.78	98.68	83.88	98.02	53.57	97.05
	1.5×10^4	82.89	94.73	68.42	90.78	11.34	81.18
	1.5×10^8	72.36	86.66	57.23	77.96	2.31	64.07
Positive control		40.83		36.73		32.22	
Negative control		0.00		0.00		0.00	

Fig.1 The percentages of effectiveness of stuck *L.fermentum* and *L.delbruekii* for different concentrations and times on the growth of *E. histolytica* trophozoites in LEM medium

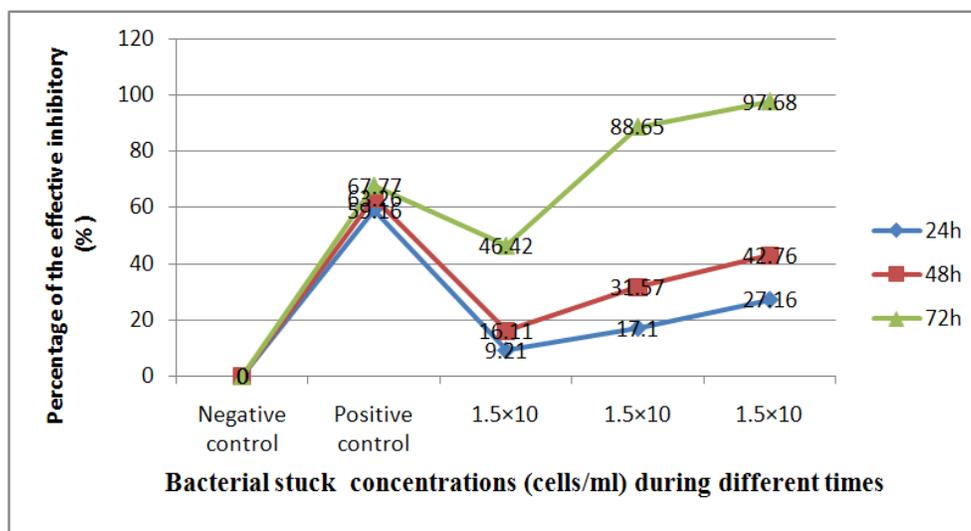


Fig.2 The percentages of effectiveness of leaky *L.fermentum* and *L.delbruekii* for different concentrations and times on the growth of *E. histolytica* trophozoites in LEM medium.

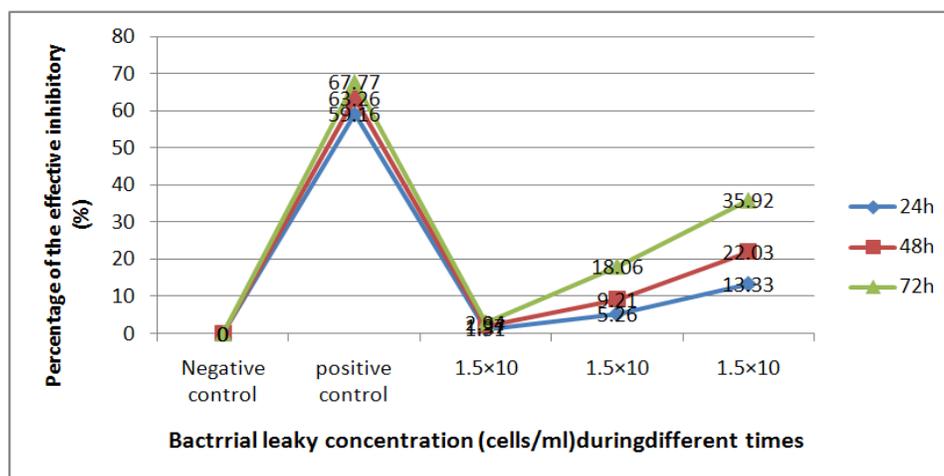


Fig.3 *Entamoeba histolytica* in culture media (LEM) that treated with (0.5) of the bacteria stuck *L.fermentum* and *L.delbruekii* (1.5×10^8) (72 hours). Showed the beginning of the the decomposition of the plasma membrane and colouration abiotic phase (trypan blue dye) (400 X).

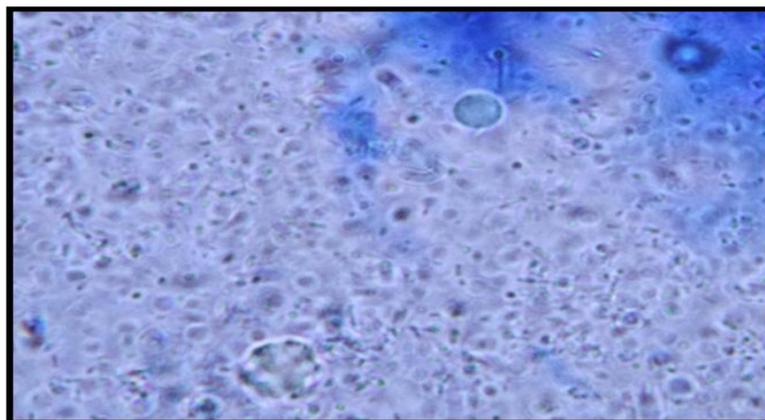
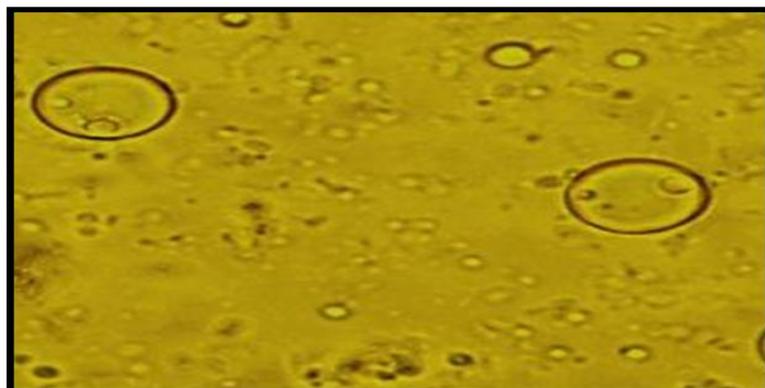


Fig.4 *Entamoeba histolytica* in culture media (LEM) that treated with (0.5) leaky *L.fermentum* and *L.delbruekii* (1.5×10^8) (72 hours). Illustrates cyste phase with binary nucleus (iodine) dye (1000 X)



The results showed that were obtained high-efficiency stuck bacteria, lactic acid concentration (1.5×10^8) after 72h incubation, to reduce the ratio of trophozite of *E. histolytica in vitro*, it recorded a therapeutic efficiency was (97.68%) of the bacteria *L.fermentum* and *L.delbruekii*. This was the highest brought by (Mohamed *et al.*, 2015) in his study of the effect of *Lactobacillus salivarius* on trophozite of *E. histolytica in vitro* and *in vivo* by the use of bacteriocins and active substances raised from bacteria, While the results have agreed and approach with brought by (Ahmed, 2012) when using different concentrations of different types of milk on the growth on trophozite of *E. histolytica in vitro*. As it recorded each of cow's milk and soybean

milk and coconut milk concentration of 10% therapeutic efficiency was (99.7%, 99.5%, 96.2%), respectively, after 48h incubation. Clarify (Ohashi *et al.*, 2003) that Lactoferrin mammals found in milk, which is secreted by the lactic acid bacteria play a role in reducing the infection, because of the ability of inhibition of the enzyme cycteine protease found in bacteria and viruses as well as some parasites. Explained (Ramos *et al.*, 2005) that the cause of this effect is the direct inhibitory damage of the plasma membrane of the bacteria. Lactoferrin is known as a single chain of glycoprotein with a molecular weight of 80KDa and produced from the mammary gland and neutrophil blood cells, and works of iron chelates irregularities outside a cell in the intestinal

surface this was confirmed (León-Sicairos *et al.*, 2005). This is consistent with the findings of a current study of stuck and filtrates effect of lactic acid bacteria against trophozoite of *E. histolytica*.

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