A PROBIOTIC APPLICATION OF *Lactobacillus acidophilus* HT1 FOR THE TREATMENT OF SOME SKIN PATHOGENS

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ABSTRACT

The present study was designed to investigate the possibility of using *Lactobacillus* as a skin probiotic. For this purpose, 63 isolates of *Lactobacillus sp.* were collected from dairy products and human mouth samples. The isolates were screened for bacteriocin production by primary and secondary screening against three common skin pathogens. The results showed that the isolate *Lactobacillus* HT1give the highest productivity of bacteriocin (320AU/ml), the efficient isolate was identified through molecular detection using 16S ribosomal RNA gene. In addition, result showed that *L. acidophilus* HT1 was un encapsulated; has no capability to produce biofilm and was susceptible to amoxicillin–clavulanic acid, vancomycin and chloramphenicol. Moreover, the infectious dose of *L. acidophilus* HT1 was studied and results revealed that mice were remained in a healthy condition including all vital processes such as breathing, consuming food and water as well as external and anatomical appearance. Next, an emul gel formula contained *L. acidophilus* HT1 biomass was prepared which already examined *in vitro* and *in vivo*. Our results confirmed the efficacy of the formula via inhibiting the growth of pathogenic isolates and through several points included: viability of *L. acidophilus HT1* cells, its ability to produce bacteriocin, and its releasing from the formula to the external environment without trapping or interaction among bacteriocin and any component of formula. *In vivo* results approved the affectivity of *L. acidophilus* HT1 biomass to treat the wounds infected with different bacterial pathogens during seven days after the onset of treatment compared with the control groups.

Key words: bacteriocin, emul gel formula, skin disease, bacteriocin application.

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استخدام بكتريا *L. acidophilus* HT1 كمعززات حيوية لعلاج بعض ممرضات الجلد

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المستخلص

هدفت الدراسة الحالية الى عزل بكتريا حامض اللاكتيك ودراسة امكانية استخدامها كمعززات حيوية لجروح الجلد. حيث تم عزل 63 عزلة من بكتريا حامض اللاكتيك من 160شملت(عينة من الالبان ومنتجاتها ومن فم الانسان). تم التحري عن قدرة هذه البكتريا في انتاج البكتريوسين من خلال الغربلة الاولية والثانوية ضد ثلاث انواع شائعة من بكتريا الممرضات الجلدية, وقد وجد ان عزلة بكتريا حامض اللاكتيك HT1 هي الاكفأ وقد اعطت اعلى فعالية للبكتريوسين تقدر ب 320وحدة/ مل. شخصت هذه العزلة الكفوءة من خلال التشخيص الجزيئي باستخدام جين الحامض النووي الرايبوسومي 16 . بالاضافة الى ذلك تم التاكد من كون العزلة HT1 غير محاطة بكبسول وليس لها القدرة على تكوين الغشاء الحيوي وجد انها حساسة لمضاد الاموكسيلين – كلافولانك, فانكومايسين والكلورمفينكول, كما تم التاكد من جرعة الاصابة لهذه العزلة وجد بقاء الفئران المختبرية بحالة صحية جيدة من خلال مراقبة العمليات الحيوية مثل التنفس واستهلاكها للطعام بالاضافة الى مظهرها الخارجي تم التأكد من المظهر التشريحي ايضا مقارنة بنموذج السيطرة. بعدها تم تحضير تركيبة الجل المستحلب التي تحتوي على الكتلة الحيوية لبكتريا حامض اللاكتيك اسيدوفلس HT1 وتم التأكد من فعاليتها مختبريا وعلى الحيوانات المختبرية, والنتائج اكدت فعالية هذه التركيبة من خلال: حيوية هذه البكترية ضمن التركيبة , قدرة البكترية على انتاج البكتريوسين ضمن التركيبة وانتاجها الى المحيط الخارجي بدون تداخل البكتريوسين مع مكونات التركيبة المستخدمة , نتائج استخدام تركيبة الجل المستحلب المحتوي على الكتلة الحيوية للعزلة المختارة على الحيوانات المختبرية اعطت فعاليتها في علاج الجروح المصابة بمختلف بكتريا الجلد الشائعة خلال سبعة ايام بعد بدء العلاج مقارنة بالمجاميع التي لم تعالج .

الكلمات المفتاحية: امراض الجلد، البكتريوسين, تركيبة الجل المستحلب، تطبيقات البكتريوسين.

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INTRODUCTION

Probiotics are single strain or a group of microorganisms having an array of beneficial effects in the human such as reducing inflammation, speeding the wound healing process and strengthening the immune system (12).This is usually achieved through a different of mechanisms such as the production of inhibitory substances like bacteriocins or acids, blockage of pathogen adhesion, antioxidant activity and nutrient competition (7, 11). Probiotics have been vastly marketed and consumed, mostly as functional foods or dietary supplements. They exert their action through enhancement of the gut barrier, epithelial repair and modulation of the immune response (12). There are numerous microorganisms used as probiotic such as *Bifidobacterium, Bacillus* species and the yeast *Saccharomyces cerevisiae* and *Lactobacillus*. *Lactobacillus* are generally recognized as safe (GRAS) and therefore, can be used as probiotics (12) . The antibacterial activity of probiotic *Lactobacilli* act against different pathogenic bacteria through multifunctional ways by secreting antimicrobial(organic acids, bacteriocins, H_2O_2, lactic acid and other), counteracting the spread within the colonized body or competing for nutrients and binding sites (11). Our skin is populated by billions of numerous bacteria. The skin and outer tissues are in constant contact with the environment, microbes have easy enter to colonize these areas of the body. There are many microorganisms associated with skin infections (atopic dermatitis (AD), eczema, acne and burns polluted) like *Propionibacterium acnes, Staphylococcus aureus, Staphylococcus epidermidis, Pseudomonas aeroginosa*, *Corynebacterium etc* (15). Experimental studies have shown that probiotics exert specific influences in the dermatology by helping preventing and treating skin conditions, including bacterial infections, psoriasis, dermatitis, the external signs of aging, acne, rosacea and yeast. Totally, probiotics exert their health effects to the skin indirectly through dietary supplementary formulations and intestinal microflora improvement or directly through cutaneous formulations (9). The aim of the current study was to utilize a safe and bacteriocin-producing isolate of *Lactobacillus* as probiotic against some common skin pathogenic bacteria via introducing it in a suitable pharmaceutical formula.

MATERIALS AND METHODS

Bacterial isolation and identification: A total of 160 samples were collected; 50 from human and 110 samples from dairy product (20) during the period from January/ 2018 to April/ 2018. Swabs were streaked in MRS agar and then incubated at 37ºC for 48hrs in microaerophilic conditions then re-cultured as single colony in MRS agar in the same conditions (21). The bacterial isolates were identified using some biochemical tests and cultural characteristics as described by Bergey's manual of Systematic Bacteriology (16).

creening of isolates for bacteriocin production

The *Lactobacillus* isolates were subjected to a screening process in order to select the higher bacteriocin producing isolate that can be used for further experiments. Three clinical isolates were used as indicators to detect bacteriocin production which all are described as common skin pathogens or skin microbiota: *Pseudomonas aeruginosa* (burn isolate), *Staphylococcus aureus* (skin injury isolate) and *Staphylococcus epidermidis* (healthy persons skin). Agar plug diffusion method was used in primary screening based on the method described by Abd and Luti (1). The zones of inhibition around the bacterial. isolates were used as a measure of antibacterial activity of each isolate Then, isolates showed antimicrobial activity were selected for secondary screening by well diffusion assay which was used to evaluate the production of bacteriocin of each isolate (1,22).

Determination of bacteriocin activity

Well diffusion assay was used to evaluate the production of bacteriocin of isolates as follows:

Amount of 20 ml of MRS broth were inoculated with 2% (10^8 cells/ml) of an overnight culture of each isolate. Then, tubes were incubated in microaerophilic condition for 24 hrs at 37°C. After incubation, the culture broth was centrifuged at 10000 rpm for 15min and the cell-free supernatant (CFS) was

collected and filtered with 0.22µmMillipore filter paper under sterile conditions (29) . Several drops of 1 N NaOH were added to (CSF) until reach to neutral pH to neutralize the effect of organic acid thereafter 10µl catalase solution were added to avoid the H_2O_2 activity. Bacteriocin activity was detected by dilution assay of CFS of each isolate against *Pseudomonas aeruginosa* using agar well diffusion assay by prepared two fold dilution series of CFS**.** The highest dilution producing an inhibition zone (DF) reflected the strength of bacteriocin activity. The bacteriocin activity which is known as arbitrary unites (AU) was determined using the following equation (5,4):

$$\mathbf{AU/ml} = \frac{\mathbf{1}}{\boldsymbol{DF}} \mathbf{X} \frac{\mathbf{1000}}{\boldsymbol{volums\ spotted\ in\ \mu l}}$$

Identification of selected isolate by using 16S ribosomal RNA

The selected bacterial isolate wasas subjected to nucleic acid extraction by using commercial DNA extraction kit (Presto Mini-DNA Bacteria Kit. Geneaid Biotech Ltd. Taiwan). The extracted DNA was estimated by Nanodrop and UV-spectrophotometer device (ACTGene avans,Taiwan) at two wave length 260/280nm. The PCR primers based on 16S ribosomal RNA gene was designed using NCBI Gene sequence data base (AB714493.1) which were provided from Macrogen Company (Korea).

Oligonucleotide Primer	Nucleotide sequence 5'-3'	Amplicon size
16SrRNA gene	CCGTAAGAAGTTGAG TGGCG ACTCTCACTCGTGTT CTTCTCT	387bp

PCR master mix (MaximePCR Premix Kit, iNtRON. Korea) prepared using master mix reagent and performed based on the company instructions. PCR products were examined by electrophoresis (Atta, Korea) with a 1% agarose gel (Promega, USA) and the 16S rRNA PCR product of *Lactobacillus sp.* isolate was purified from agarose gel by using (EZ EZ-10 Spin Column DNA Gel Extraction Kit, Biobasic. Canada). The purified 16S rRNA gene PCR product samples were sent to Macrogen Company in Korea for performed the DNA sequencing using 16S rRNA forward primer by AB DNA sequencing system.

Antimicrobial sensitivity

The susceptibility of *Lactobacillus* isolate to different antimicrobials was determined according to Kirby-Bauer disc diffusion method (6). The antibiotics disc used were Amoxicillin,Amoxicillin/clavunic acid, Streptomycin, Vancomycin, Clindamycin, Gentamicinand chloramphenicol.

Biofilm formation

The ability of the selected *Lactobacillus* to form biofilm was detected via two methods as follow: Congo red agar method**,** this method was achieved according to Freeman, *et al***.,** (13). *Lactobacillus sp*. isolate was streaked on congo red agar and then incubated for 24-48hrs at 37C◦. After incubation, the appearance of sparkle dark colonies indicates biofilm formation. Microtiter plate method*:* briefly *Lactobacillus* isolate was grown in MRS broth with 1% glucose for 48hrs at 37C◦ in microaerophilic condition. An amount of 180µl of sterile TSB was added to each well in a microtiter plate, and then 20 µl of 48hrs grown *Lactobacillus* was added. The broth in the well was mixed 10 times by pipette and then incubated for 72hrs at 37C◦ without shaking. After incubation, the supernatant was removed, and each well was washed 3 times by phosphate buffer. Aliquots of 200 µl of 1% crystal violet were added for 15 minutes. The wells were washed 3 times with phosphate buffer and then dried by air for 30 minutes. An amount of 200 µl ethanol (96%) was added for 15 minutes. Sterile medium was used as a negative control. The result was read using ELISA reader **(**Huma reader HS, Germany) at 630nm (17).

Capsule detection

Capsule staining was achieved using nigrosin stain (28).

Infectious dose (ID) (*In vivo* tests)

The infectious dose was determined according to Welkos and Alison (32)**,** Twenty albino mature mice were divided into three groups, the first one was considered as control and included four mice, the two other groups were specified to different treatment, each one included eight mice. Serial dilution of the selected bacterial cells suspension was prepared to obtain two concentrations:10^8, 10^3 cell/ml. The infectious dose was determined as follow: Group1**:** The mice were injected intra-

peritoneal (IP) with 1ml of high dose of bacterial isolate (HT1) suspension (10^8). Group2**:** the mice were injected IP with 1ml of low dose of bacterial isolate suspension 10^3), Group3: The mice were injected with 1 ml of PBS.

Pharmaceutical formula including lived *Lactobacillus* biomass

Emul gel (100 gm) was prepared as described by (25) with some modifications as follow: The formula included two phases:

Phase A: Amount of 1.08 gm of span 60 was dissolved in 30 ml of olive oil and mixed using magnetic stirrer at 45°C. Then, the mixture was allowed to cool. 0.5 gm of carbopol 934 was dispersed in the oily mixture, and 0.1 of methyl paraben was added with stirring.

Phase B (the aqueous phase*):* 5gm of Propylene glycol was added to 59.8 ml D.W. Phase A was slowly added to Phase B and emulisified using mixer for 10 min at low speed. Next, the speed was gradually increased until homogenization. 1gm hydroxy propylen methycellulose (thickener agent) was added with mixing at low speed for 45 min. Aliquot of 10ml of optimized medium (modified nutrient broth) were added to the formula and then incubated under optimum conditions at 37°C, pH 6.4 for 24hrs. After incubation, the number of cells were adjusted with 0.5 McFarland Standard tube to be obtained approximately 1.5×10^8 cell/ ml. Thereafter, biomass was collected using centrifuge at 12000 rpm for 15min, and then a known amount of the optimized medium was added to the precipitant. This cells suspension was kept as usable biomass to be mixed later with the formula (33). The formula including biomass, medium with all compound in (Phase A and Phase B) which mixed very well until homogenization. The pH was adjusted to 6.4 by adding triethanolamine in drop wise until gelling emulsion was formed. The formula was stored in close container at 4ºC. the *L. acidophilus* HT1 biomass was prepared with different concentration (10^3, 10^4,10^5 ,10^6, 10^7, 10^8) were used to determine the suitable biomass concentration that lead to inhibit selected pathogenic bacteria within emul gel formula and tested using *In vitro* evaluation of emul gel formula**.**

***In vitro* evaluation of emul gel formula**

Three multidrug resistant bacterial isolates included; *Staphylococcus aureus, Klebsiella sp., Pseudomonas aeruginosa* were used as indicators *in vitro* and *in vivo* experiments. These indicators were prepared in brain heart infusion broth and incubated at 37°C for 24hrs. Then, the number of cells was adjusted according to McFarland tube (0.5) which is equal to 1.5×10^8 cell/ml at 600nm. The antibacterial activity of the emul gel formula containing active *Lactobacillus* cells was evaluated using agar well diffusion method (26).

***In vivo* evaluation of emul gel formula**

This experiment was conducted to improve the capability of selected bacterial isolate as a probiotic material in a new way of external treatment on the skin. The experiment was achieved with rabbits as follow: 12 local white rabbits were divided into 3 groups each group included 3 animals as treated animals and the fourth one was considered as control. Each group was specified for one indicator bacteria. After adaptation period (3 days), rabbits' shoulder and thigh regions were shaved and injured using a scalpel. Then, each rabbit group was infected with one of bacterial indicator and already grouped as 1,2 and 3 for *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Klebsiella* respectively. Each group of experiment were treated as follow: one of rabbit are treated at the onset time after infection (treatment directly after infection), this group was considered as prophylactic group. In addition, another animal was treated with final formula containing *Lactobacillus* after few hours of infection (treatment group). The last one was treated by the formula components only without active material (*Lactobacillus* biomass). The experiment scheduler set ending time was based on the time of complete healing and the formula was applied twice daily, the results were monitorized after 7 days of treatment.

RESULTS AND DISCUSSION

A total of 160 samples were collected from human mouth of healthy individuals and from dairy products. The isolates were identified as related to the genus *Lactobacillus* using MRS media as selective media and via number of morphological and biochemical tests according

to Bergey's manual of Systematic Bacteriology (18, 27). The screening process (primary and secondary) by agar pluge diffusion method and well diffusion method respectively was used to highlight the antagonism between different isolated strains through inhibit the growth of common skin bacterial species were chosen to be used for further experiments in this study. In addition, *Pseudomonas. aeruginosa* was chosen to be used as indicator for the next experiment. Based on results, 17 isolates were able to produce bacteriocin active against the three indicators used in this experiment (*Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Staphylococcus epidermidis*) with different diameter of inhibition zones and therefore were selected for the secondary screening. Next, the Seventeen isolates showed antimicrobial activity in the primary screening were selected for secondary screening by well diffusion assay. According to the results presented in Table 1, the isolate *Lactobacillus* HT1 was the most active isolate for bacteriocin production with 320 AU/ml against all the indicators bacteria. Therefore, this isolate was chosen to be used for further experiments in this study. In addition, *Pseudomonas aeruginosa* was chosen to be used as an indicator for the next experiments. The (PCR) amplification products of 16S ribosomal RNA gene were examined by agarose gel electrophoresis and the results revealed that the amplified DNA fragment in line (1) was approximately387bp in length (Figure 2) . Thereafter, the DNA sequencing analysis was conducted by using phylogenetic tree UPGMA method (MEGA 6.0 version), Multiple alignment analysis based Clustal W alignment analysis, and NCBI- BLAST for homology sequence identity. As can be seen in Figure 3, results revealed that this DNA is belong to *Lactobacillus acidophilus* with 99.18% similarities.

Table 1. Bacteriocin production by *Lactobacillus* isolates in secondary screening

Isolate	Bacteriocin activity (AU/ml)		
	S. aureus	*P. aeruginosa*	*S. epidermidis*
HT 1	320	320	320
HT 2	320	320	160
HT 4	20	20	20
HT 5	20	40	20
HT 6	40	20	20
HT 8	40	20	20
HT 12	40	40	160
HT13	40	40	20
HT14	40	40	20
HT15	40	20	160
HT16	20	20	320
HT17	40	320	20
HT28	40	320	20
HT29	20	320	40
HT30	320	320	40
HT35	320	320	40
HT152	320	320	20

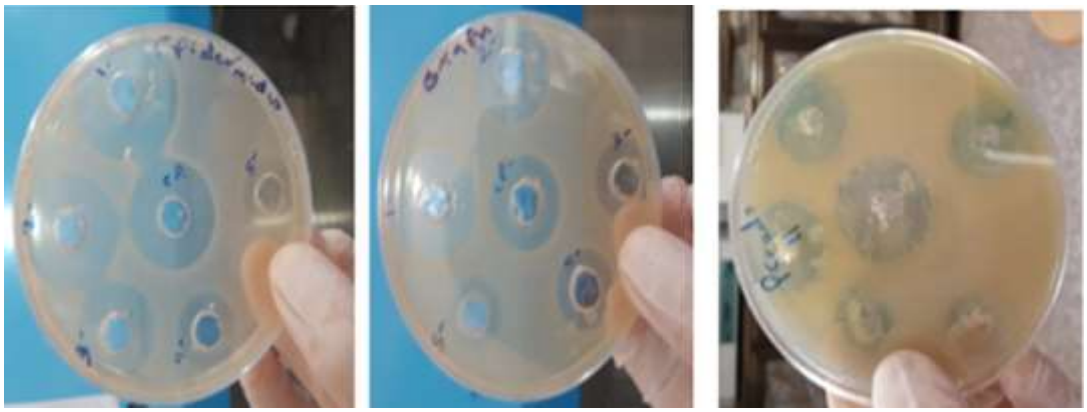

Figure 1. Secondary screening of *Lactobacillus* isolates against *S. epidermidis S. aureus* and *P. aeruginosa* respectively

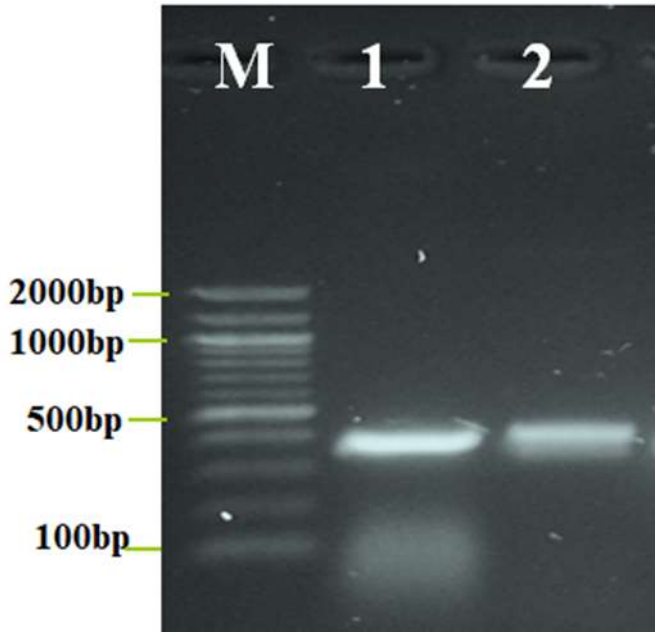

Figure 2. Agarose gel electrophoresis image show the PCR product of 16S ribosomal RNA gene used in the molecular detection *lactobacillus* isolate HT1. Where M: Marker (2000-100bp), lane (1-2) *lactobacillus* isolate HT1

Lactobacillus sp. isolate No.1 16S ribosomal RNA gene

Sequence ID: Query_21716 Length: 365 Number of Matches: 1

Range 1: 1 to 365 Graphics ▼ Next Match ▲ Previous Match

Score	Expect	Identities	Gaps	Strand
658 bits(356)	0.0	362/365(99%)	0/365(0%)	Plus/Plus

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Query  1    GCCCCGCGGTGCATTAGCTAGTTGGTAGGGTAACGGCCTACCAAGGCAATGATGCATAGC  60
Sbjct  1    .........A.....................................................  60

Query  61   CGAGTTGAGAGACTGATCGGCCACATTGGGACTGAGACACGGCCCAAACTCCTACGGGAG  120
Sbjct  61   ............................................................  120

Query  121  GCAGCAGTAGGGAATCTTCCACAATGGACGAAAGTCTGATGGAGCAACGCCGCGTGAGTG  180
Sbjct  121  ............................................................  180

Query  181  AAGAAGGTTTTCGGATCGTAAAGCTCTGTTGTTGGTGAAGAAGGATAGAGGTAGTAACTG  240
Sbjct  181  ............................................................  240

Query  241  GCCTTTATTTGACGGTAATCAACCAGAAAGTCACGGCTAACTACGTGCCAGCAGCCGCGG  300
Sbjct  241  ............................................................  300

Query  301  TAATACGTAGGTGGCAAGCGTTGTCCGGATTTATTGGGCGTAAAGCGAGCGCAGGCGGAA  360
Sbjct  301  ............................A.................G.........  360

Query  361  GAATA  365
Sbjct  361  .....  365
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Figure 3 . Multiple sequence alignment analysis of the partial 16S ribosomal RNA gene sequence for local *Lactobacillus sp* HT1. with NCBI-Blast *Lactobacillus spp*. 16S ribosomal RNA gene by using (NCBI-BLAST analysis tools). The multiple alignment analysis similarity (*) and differences in 16S ribosomal RNA gene nucleotide sequences

Evaluation of the selected *Lactobacillus acidophilus* HT1 virulence factors

Based on the literature, the factors that must be addressed in the evaluation of safety of probiotics include mainly the pathogenicity, infectivity, and virulence factors comprising toxicity, metabolic activity (10). The intrinsic properties of the microbes, provide some methods for assessing the safety of lactic acid bacteria via using an *in vitro* and *in vivo* studies. In order to assess the safety of the selected isolate, *Lactobacillus acidophilus* HT1, four tests were investigated (antibiotic sensitivity, Biofilm formation, Determination of infectious dose (ID) and Capsule detection) which all revealed the safety of *L. acidophilus* HT1isolate (10). The results presented in Table 2 revealed that *L. acidophilus* HT1 isolate was susceptible to amoxicillin–clavulanic acid mixture and followed the same pattern for amoxicillin alone. In addition, *L. acidophilus* HT1 isolate was very sensitive to vancomycin which belong to the cephalosporins. (19) Hamilton-Miller and Shah found in their study that *L. acidophilus* and *L. delbreuckii* strains were sensitive to

vancomycin, while all the other strains mainly *L. Rhamnosus* were resistant. Moreover, the selected isolate showed sensitvity to chloramphenicol. Whereas this isolate showed high resistant to clindamycin, streptomycin and gentamycin. This result is agreed with the results of Ammor *et al.* (2) who mentioned that *Lactobacillus* was commonly sensetive to antimicrobials that inhibit protein synthesis such as chloramphenicol and tetracycline, but more resistant toward aminoglycosides such as streptomycin and gentamicin. Micro-organisms produce an extracellular polymeric substance responsible for the flow of nutrients inside biofilm matrix**,** thereby interfering with antimicrobial therapy (8). Thus, detection of biofilm formation can help prevent potentially persistent infections and fatal (30). In this study, two methods were used to detect the ability of the selected isolate, *L. acidophilus* HT1, to form biofilm; Congo red agar and Microtiter plate (Tissue Culture Plate method). The result of culturing selected *Lactobacillus acidophilus* HT1 on Congo red agar showed that, the bacterial isolate has no capability to produce biofilm. Freeman *et al*., (13) documented that non-biofilm producer colonies remaine pink to red on Congo red agar. In addition, tissue Culture Plate (TCP) method was used to determine the degree of biofilm production (strong, intermediate and weak). The results confirmed the previous findings by Congo red method in which *L. acidophilus* HT1 had no capability to produce biofilm and these results were in general in agreement with the findings observed by Hassan *et al*., (17) and Anuradha *et al*., (3). The negative formation of biofilm by the selected *L. acidophilus* HT1 provides an evidence for the biosafety usage as probiotic. Moreover, the infectious dose of the selected *L. acidophilus* HT1 isolate was investigated according to the results of Welkos and Alison, (32). The results of this experiment were obtained after one week for inoculation doses, 10^8 and 10^3 cell / ml (i.p). All lab animals were remained in a healthy condition including all vital processes such as breathing, consuming food and water as well as external appearance. Furthermore, the anatomical results of all mice showed no grossly change in all abdominal organs and fluid the same as in the control group. Furthermore, the result showed that *L. acidophilus* HT1 was un encapsulated which can be considered as a second good trait for this isolate because capsule with biofilm formation are considered as virulence factors (10).

Table 2. Results of antimicrobials used to test the susceptibility of *L. acidophilus* HT1 isolate

Antimicrobial disks	Symbol	Concentration µg/ disk	Susceptible	Resistance
Amoxicillin	Ax	25mcg	S	
Amoxicillin/clavunic acid	Amc	(20/10) mcg	S	
Gentamicin	GM	10 mcg		R
Streptomycin	S	10 mcg		R
Vancomycin	VA	30 mcg	S	
Clindamycin	CD	2 mcg		R
Chloramphenicol	C	30 mcg	S	

Pharmaceutical formula including lived *L. acidophilus* HT1 biomass

Topical delivery drugs are a good method for local and systemic treatments and generally used in the treatment of inflammatory conditions like dermatological diseases (24). Topical application has many advantages over the predictable dosage forms, especially to avoid some serious systemic adverse effects**,** and its action occurs directly at the action site (31). The physical characteristic of prepared emul gel formula has a smooth texture and white color transparent and homogeneous with odorless. In this study, *L. acidophilus* HT1 biomass was successfully incorporated into emul gel bases formula. The viability of *L. acidophilus* HT1 cells was checked for fourteen days by daily culturing on MRS agar after preparation, and homogeneity of formula components to ensure the activity of formula . Based on results, cells have kept their viability over fourteen days. In general**,** the prepared formula emulgels are evaluated by several parameters such as pH, homogeneity, spread ability, skin irritation (10) . Both pH and homogeneity were monitored *in vitro* which revealed the stability of the formula. The prepared formula showed stability at pH (5.5–6.5) and homogeneity through fourteen days. In addition, the skin irritation test was

evaluated during the *in vivo* experiment, which confirm that the formula caused no irritation signs (redness, swelling, increasing the temperature in treated area) during all experiment period. The antibacterial activity of *L. acidophilus* HT1 biomass within emulgel preparations was investigated against *Pseudomonas aeruginosa* according to the method described by Shahin *et al*., (25). For this purpose, different concentrations of *L. acidophilus* HT1 biomass (10^3, 10^4,10^5 ,10^6, 10^7, 10^8) were used to determine the suitable biomass concentration that lead to inhibit selected pathogenic bacteria. The result showed that 10^8 CFU/ml was the best concentration as shown in figure 4 . Inhibition of *P. aeruginosa* growth reflected the efficiency of the formula through several points included: viability of *L. acidophilus HT1* cells, its ability to produce bacteriocin, and its releasing from the formula to the external environment (tested media), without trapping or interaction among bacteriocin and any component of formula that may counteract bacteriocin ability to inhibit tested bacteria. The formula with *L. acidophilus* HT1 was examined to treat three groups of rabbits infected with three types of pathogenic bacteria: *P. aeruginosa*, *S. aureus* and *Klebsiella spp* (indicators bacteria). The results of using biomass through monitoring for 7 days approved the affectivity of *L. acidophilus* HT1 biomass to treat the wounds infected with different bacterial pathogens were resulting during seven days after the onset of treatment. Complete healing for all types of induced infected wounds was occurred after seven days of treatment compared with the control groups as shown in Figure (5,6,7). Furthermore, the treatment at the onset of infection was more affective against bacterial pathogens. Therefore, this type of treatment can be considered as a prophylactic from infection. On the other hand, the formula was act as an adjuvant via helping the *L. acidophilus* HT1 to produce the beneficial compounds that lead to counteracting bacterial pathogen to grow or producing toxins or/and enzymes. These results reflected the ability of formula to spread excellently, because the therapeutic efficacy of gels or emul gel is usually depends on their spread. Emul gel spreading helps in the uniform application of the gel to the skin, thus, emul gels have excellent spreading ability which leading to a perfect quality in topical application. Furthermore, this is considered an important factor in patient compliance with treatment (23).

Figure 4. The antibacterial activity of *L. acidophilus HT1* biomass (10^8)formula against *Pseudomonas aeruginosa* (two fold dilution in each well of *L. acidophilus* HT1 biomass formula).

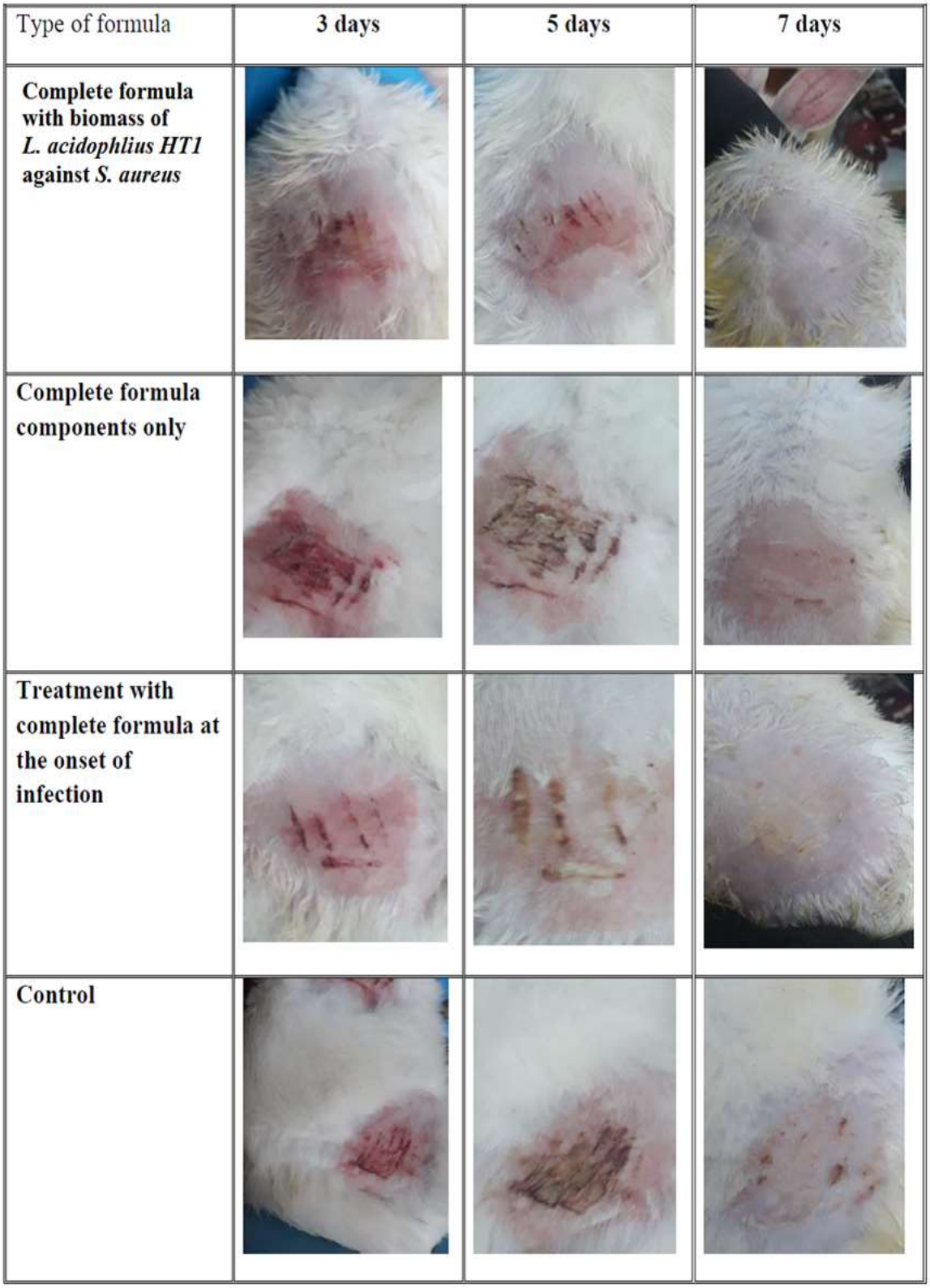


Figure 5 . *In vivo* experiment for applying *L. acidophilus HT1* biomass formula to treat a group of rabbits infected with *Staphylococcus aureus.*

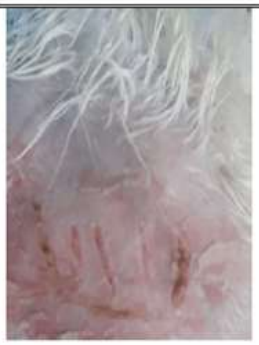
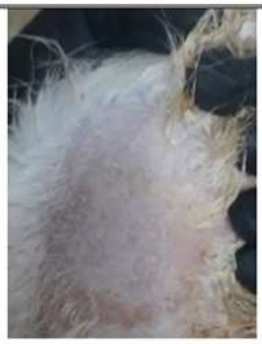
Type of formula	3 days	5 days	7 days
Complete formula with biomass of *L. acidophilus HT1* against *Klebsiella*			
Complete formula components only			
Treatment with complete formula at the onset of infection			
Control			

Figure 6 . *In vivo* experiment for applying *L. acidophilus HT1* biomass formula to treat a group of rabbits infected with *Klebsiella spp*

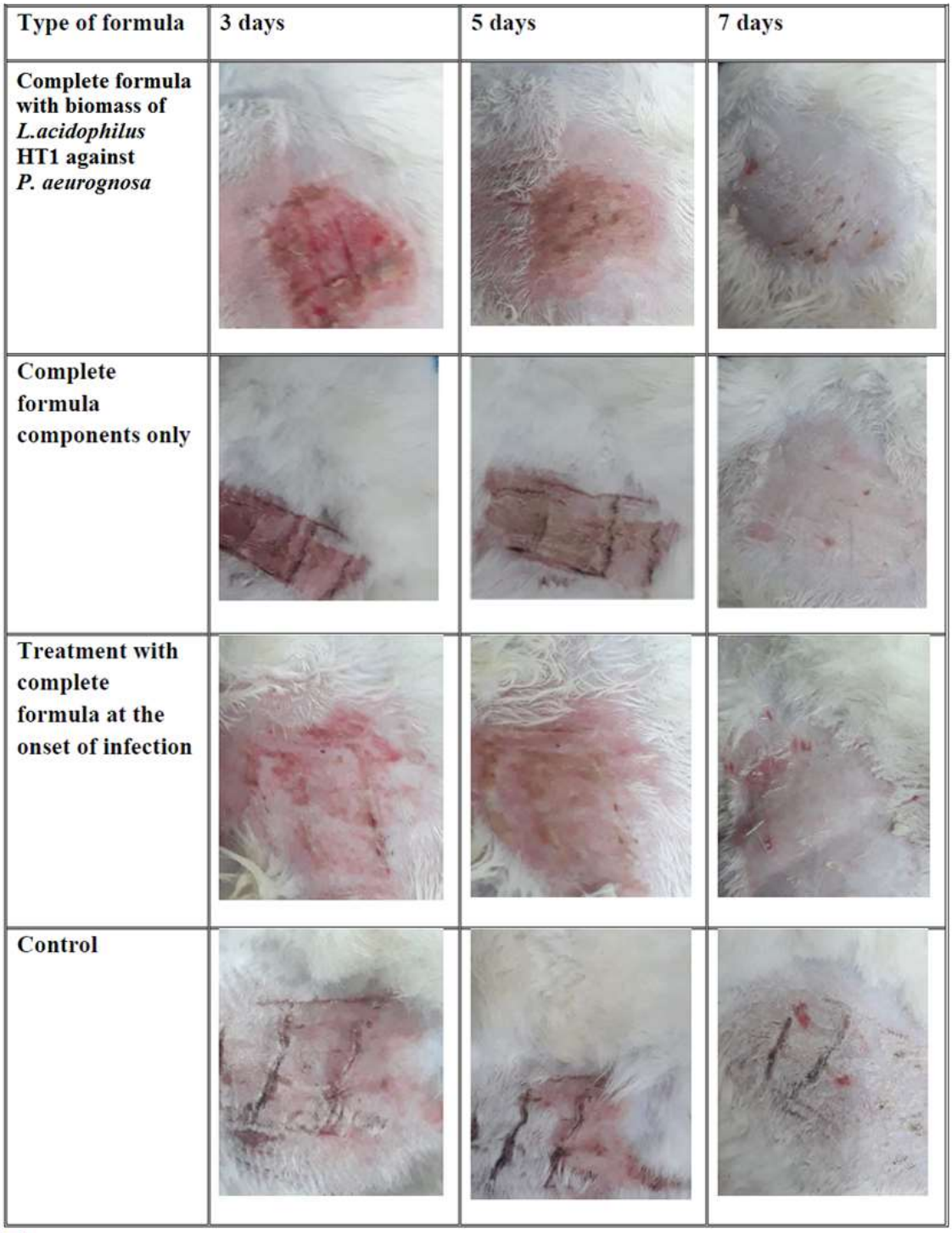


Figure 7. *In vivo* experiment for applying *L. acidophilus HT1* biomass formula to treat a group of rabbits infected with: *Pseudomonas aeruginosa*

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