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Probiotic Application of Bacteriocin-Producing *S. Epidermidis* in A Cellulosic Pad to Treat Some Skin Infections

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Abstract

The aim of this study was to evaluate the possibility of using *Staphylococcus epidermidis* cells as a probiotic to treat some skin infections. For this purpose, *S. epidermidis* Y73, which is an active bacteriocin producer and non-biofilm forming isolate, was selected among 134 skin isolates through primary and secondary screening. Tryptic soya broth was selected as the best medium to support bacteriocin production, while the optimal pH and temperature for *S. epidermidis* Y73 growth were 7 and 37°C, respectively, which were invested in the formula preparation. Furthermore, the possibility of using this isolate as a probiotic was investigated by preparing 4 potential cellulosic pads with 4 different formulae which were all subjected to an *in vitro* trial to select the one which is superior to the others in terms of supporting bacteriocin production and cells viability. The shelf life of the pad was estimated and the results showed that the cells remained vital until the 20th week. The selected pad formula was used to treat artificially induced wounds on rabbit skin. The wounds were infected with *Staphylococcus aureus*, *Kocuria rosea* and *Pseudomonas. aeruginosa*. The symptoms in both control and treated animals were recorded and, based on the results; the healing process with the presence of the *S. epidermidis* Y73 pad was significantly faster compared with that for the control. This research will serve as a base for future studies on using vital cells of *S. epidermidis* as probiotics and, hence, make a contribution to the current literature on using live cells to treat bacterial skin infections.

Keywords: Skin; *Staphylococcus epidermidis*; Probiotic; Bacteriocin

معزز حيوي من المكورات الجلدية المنتجة للبكتريوسين في ضمادة سليلوزية لعلاج بعض الالتهابات الجلدية

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الخلاصة

ان الهدف من هذه الدراسة هو تقييم إمكانية استخدام خلايا المكورات الجلدية كعزز حيوي لعلاج بعض الالتهابات الجلدية. لهذا الغرض، تم اختيار العزلة *S. epidermidis* Y73 المنتجة لبكتريوسين فعال وغير مكونه للاغشية الحيوية، من بين 134 عزلة جلدية من خلال العزلة الأولية والثانوية. تم اختيار وسط مرق الصويا كأفضل وسط لدعم إنتاج البكتريوسين وكان الرقم الهيدروجيني ودرجة الحرارة المثلى للنمو *S. epidermidis* Y73 و 37 درجة مئوية، والتي تم استثمارها في إعداد التركيبة. تم التحقق في إمكانية استخدام هذه العزلة كعزز حيوي، عن طريق إعداد اربعة ضمادات سليلوزية مع 4 تركيبات مختلفة حيث تم

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اختبارها مختبرياً لاختيار الاكفأ من حيث دعم إنتاج البكتريوسين والحفاظ على حيوية الخلايا. تم تقدير العمر الافتراضي للضمادة وأظهرت النتائج أن الخلايا احتفظت بحيويتها حتى الأسبوع العشرين. تم استخدام الضمادة المحددة لعلاج الجروح على جلد الأرنب. أصيبت الجروح ببكتريا المكورات العنقودية الذهبية ، وكوكوريا روزيا ، والزائفة الزنجارية. تم تسجيل الأعراض في كل من الحيوانات السيطرة والمعالجة واستنادا إلى النتائج ، كانت عملية الشفاء مع وجود الضمادة الحاوية على خلايا *S. epidermidis* Y73 أسرع بكثير مقارنة مع السيطرة. هذا البحث سيكون بمثابة قاعدة للدراسات المستقبلية حول استخدام الخلايا الحية لبكتريا المكورات الجلدية كمعزز حيوي ، وبالتالي المساهمة في الأدبيات الحالية حول استخدام الخلايا المايكروبية الحية لعلاج الالتهابات الجلد البكتيرية.

Introduction

Probiotics are living microorganisms having an array of beneficial effects in humans, such as reducing inflammation, speeding the wound healing process, and strengthening the immune system [1]. This is usually achieved through different mechanisms, including the production of inhibitory substances such as bacteriocins or acids, blockage of pathogen adhesion, antioxidant activity, and nutrient competition [2]. 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 [1].

There are numerous microorganisms used as probiotics, such as *Bifidobacterium* and *Lactobacillus* which are most commonly used. However, some strains of *E. coli*, *Bacillus*, and *Saccharomyces cerevisiae* are also used [3]. The antibacterial activity of probiotics acts against different pathogenic bacteria through multifunctional ways, mainly by secreting antimicrobial substances, such as organic acids, bacteriocins, H_2O_2 , lactic acid and others.

Human skin is populated by billions of numerous bacteria and due to their constant contact with the environment; microbes have easy colonization ability to various areas of the body. There are many microorganisms associated with skin infections, such as atopic dermatitis, eczema, acne and burns. These microorganisms include, for example, *Propionibacterium acnes*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Corynebacterium* [4]. Experimental studies showed that probiotics exert specific influences in the dermatology by helping the prevention and treatment of skin infections, including bacterial infections, psoriasis, dermatitis, the external signs of aging, acne, rosacea and yeast infections [5].

A wound is a tissue damage usually caused by membrane laceration that generally results in skin damage. Most chronic wounds contain more than one bacterial species which result in a synergetic effect causing non-virulent bacterial species becoming virulent and evoking host damage [6]. In the literature, investigations of the bacterial diversity in chronic wounds showed that *Staphylococcus*, *Pseudomonas*, *Peptoniphilus*, *Enterobacter*, *Stenotrophomonas*, *Fingoldia*, and *Serratia* are the most common in chronic wounds [6]. In addition, bacteria in chronic wounds were observed to develop biofilms which contribute to a delay in healing. Madsen *et al.* [7] reported that chronic wounds are colonized by various bacterial populations, which contribute directly and indirectly to chronic phenotypes of wounds. Colonizing bacteria such as *Staphylococcus epidermidis* and *Corynebacterium* are normally present on the skin and usually prevent pathogenic bacteria colonization by immune orientation and competition on resources as well as bacteriocin production, which are all criteria that are present in the selected bacterial strains. The aim of the current study was to utilize a safe and bacteriocin-producing isolate of *S. epidermidis* as a probiotic against some common skin pathogenic bacteria via introducing it in a suitable pharmaceutical formula.

Materials and Methods

Collection of *S. epidermidis* isolates

Skin samples (swabs) were collected from healthy individuals in order to seek a potential bacteriocin producing normal microbiota of *Staphylococcus epidermidis*. First, the swabs were placed in tubes containing 5 ml of brain heart infusion broth as an enrichment medium. After 24 hrs of incubation at 37°C, serial dilutions were prepared in normal saline and a swab from the dilution of 10^{-5} was applied on mannitol salt agar. Then, after 24 hrs of incubation at 37°C, the potential candidates for being *S. epidermidis* which were fitting the morphological criteria were selected and sub cultured on MS agar

to ensure purity. These isolates were identified through biochemical tests and cultural characteristics as described by Bergey's manual and using VITEK system.

Screening of *S. epidermidis* isolates for bacteriocin production

Primary screening

Well diffusion assay was used to evaluate the production of bacteriocin, as follows [8]: Universal tubes containing 5ml of broth were inoculated with 2% of an overnight culture of the isolate containing approximately 1×10^8 cells/ml and then incubated for 24 hrs at 37°C. After incubation, the cell-free supernatant (CFS) was collected and assayed for the presence of bacteriocin using agar well diffusion assay, as follows: 100 µl of an overnight growth culture of the indicator bacteria (4 different isolates of *S. aureus* and 2 isolates of *Pseudomonas aeruginosa*) containing approximately 1×10^8 cells/ml was mixed with 25 mL of a sterile Mueller Hinton agar and then poured into sterile Petri dishes. Wells of 6 mm diameter were cut and 100 µl aliquots of the filtered CFS were dispensed in each well. Then, plates were incubated for 24 hrs at 37°C [9, 10].

Secondary screening (Bacteriocin activity assay)

Bacteriocin activity was determined for the selected isolates from the primary screening by using the critical dilution assay which is similar to the minimum inhibitory concentration technique (MIC) for antibiotic assessment [11]. In addition, the biofilm detection for *S. epidermidis* isolates was integrated in the screening by using the microtiter plate assay [12,13,14].

Determination of best production medium for *S. epidermidis*

Different nutritional media (nutrient broth, brain heart infusion broth, tryptic soy broth, modified brain heart infusion broth, mueller hinton broth and Thomas medium) [15] were tested in order to select the medium that supports maximum bacteriocin production by the selected *S. epidermidis* isolate [16].

Characterization of *S. epidermidis* as a probiotic

Antibiotic susceptibility testing

Antibiotic susceptibility was conducted using the disc diffusion method [17] on a Mueller-Hinton agar which was previously seeded with the selected *S. epidermidis* Y37.

Determination of optimal pH and temperature for *S. epidermidis* Y37 growth

Six universal tubes containing 5 ml of tryptic soy broth with pH values ranged from 4 to 9 were inoculated with the same cell concentration of the selected isolate (10^8 cell/ml) and then incubated for 24hr at 37°C for 24 hrs (the tubes were weighed empty before the procedure). Afterwards, the tubes were centrifuged at 10000 rpm for 10 min, the cell free supernatant was discarded, and the tubes were re-weighed with the biomass within. The difference in weight was marked as the biomass [18]. The same procedure was followed to determine the optimal temperature via incubation at different temperatures: 25, 30,35,37,40 and 45°C.

Biofilm formation assay

The Biofilm detection for *S. epidermidis* isolates was achieved by using the microtiter plate assay based on the method described earlier [12, 13]

Probiotic preparation

Pad design

Four pads of thick cellulosic filter paper with 1cm width and 3 cm length were prepared. Four potential formulae were used as follows [19]

1. 150 µl TSB containing *S. epidermidis* at a concentration of 1×10^8 cells/ml.
2. 100µl TSB containing *S. epidermidis* at a concentration of 1×10^8 cells/ml plus 50 µl petroleum jelly,
3. 100µl TSB containing *S. epidermidis* at a concentration of 1×10^8 cells/ml plus 50 µl glycerol,
4. 100µl TSB containing *S. epidermidis* at a concentration of 1×10^8 cells/ml plus 25 µl petroleum jelly and 25 µl glycerol.

Then, all pads were placed on MSA which was previously seeded with 1×10^4 cells/ml of *S. aureus* as an indicator in order to investigate the antagonistic capacity of the tested pads. The superiority of the selected pad was determined based on beneficial criteria obtained in terms of competition capacity and shelf life.

Estimation of expiration date of the probiotic pad

Expiration date of the pad was estimated according to the strategy proposed by Capen *et al.* [20]. A twenty-weeks trial was conducted to estimate the shelf life of the pad and investigate how long the

cells will remain vital in the selected formula. This was achieved by preparing 20 pads in sterile conditions with the selected formula. The tubes were then sealed with parafilm and preserved in sterile tubes at 4°C; this point was considered as the zero time. Along the 20 weeks, every seven days, one pad was picked out from the batch and placed on MSA plate which was then incubated for 24hr at 37°C to check the vitality and purity of *S. epidermidis* cells.

In vivo application of the probiotic pad

Six local rabbits were used in this experiment, which were divided into 3 groups each with 2 animals; one was the treatment group and the other was considered as a control. Each animal was specified for one indicator pathogenic bacteria. Animals were shaved one day prior to the infection using scissors and razor at the shoulder site. The animals were housed in small cages (one animal per cage) and allowed for food and water. Incision was made with 2 to 2.5 cm in length using a lancet in the shoulder. Then, in each rabbit group, the rabbits were marked as 1, 2 and 3 which were infected with *S. aureus*, *P. aeruginosa*, or *K. rosa* respectively. The infection was achieved via introducing the pathogens into the wound using a swab at a concentration of 1×10^4 . The *S. epidermidis*-containing pad was fixed on the wound of the treated group of animals using wound patch (pad 3). It was placed at the zero time after introducing the inoculum and wrapped with bandage for extra fixation. Every 24 hrs, the old pad was removed and replaced with a new one. The trial was meant to last until full recovery was retrieved and this was accomplished within 7 days.

Results and Discussion

In this study, 134 *S. epidermidis* isolates from skin samples were obtained from healthy individuals. The morphological and biochemical examinations of *S. epidermidis* isolates were achieved based on Bergey's manual of systematic bacteriology [21]; catalase, blood hemolysis and coagulase tests are useful to identify *S. epidermidis* and, therefore, these tests were performed for all isolates (data not shown). Thereafter, *S. epidermidis* isolates were subjected to a screening process in order to select the higher bacteriocin-producing isolate that can be used in this study. The screening was achieved in TSB medium and for more reliability all isolates were cultivated under the same conditions in terms of inoculum size, cell number, pH and incubation period. Four isolates of *S. aureus* and 2 isolates of *Pseudomonades aeruginosa* were used as indicator strains in order to determine the antibacterial activity of *S. epidermidis* isolates. These indicators were chosen on the bases that all of them are important skin pathogenic bacteria.

The primary screening (fig 1) was conducted using the well diffusion method to discriminate the isolates possessing the capacity of producing bacteriocin. Based on the results, 22 isolates were selected which were then subjected to a secondary screening program. Since *S. epidermidis* is not a text book probiotic bacteria, the secondary screening needs to be modified from the traditional ones. Therefore, in addition to the bacteriocin activity determination, the biofilm forming capacity of the isolates was integrated in this step for the value it has as a virulence factor [22]. Tissue culture plate method (TCP) was used to evaluate the biofilm forming ability and the results from the previous two factors are listed in Table-1.

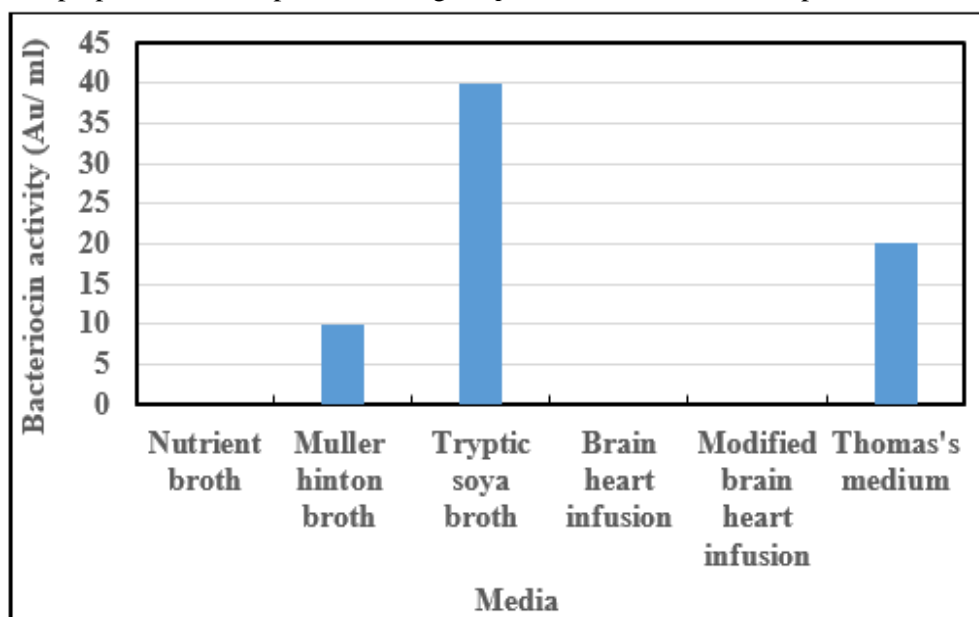


Figure 1-Primary screening of *Staphylococcus epidermidis* isolates for bacteriocin production by well diffusion method to detect Bacteriocin production.

Table 1-The secondary screening of *Staphylococcus epidermidis* isolates for bacteriocin production, bacteriocin activity and biofilm formation.

Isolate	AU/ml value	Biofilm detection
Y11	10	Non-adherent
Y51	10	Non-adherent
Y34	10	Non-adherent
Y72	10	Non-adherent
Y99	10	Non-adherent
Y53	10	Non-adherent
Y9	20	Non-adherent
Y69	10	Non-adherent
Y14	10	Non-adherent
Y54	10	Non-adherent
Y104	10	Weakly adherent
Y10	10	Non-adherent
Y87	20	Non-adherent
Y102	10	Non-adherent
Y73	40	Non-adherent
Y92	10	Non-adherent
Y16	10	Non-adherent
Y114	10	Non-adherent
Y75	10	Non-adherent
Y111	40	Weakly adherent
Y110	10	Weakly adherent
Y27	10	Non-adherent

By combining the data from both primary and secondary screening, including the wide range of activity against indicators and high bacteriocin activity as well as being non-biofilm producer, it was logical to select the isolate Y73 as the elected candidate among the other isolates. Next, six media were used in this experiment in order to investigate the best medium that can support maximum bacteriocin production. As can be seen in Figure-2, TSB was the best medium to support bacteriocin production with an activity of 40 AU/ml. Therefore, this medium was used in the next experiments as well as in the preparation of the pad containing *S. epidermidis* Y73 cells as a probiotic.

**Figure 2**-The production of bacteriocin by *Staphylococcus epidermidis* Y73 in different media

Characterization of *S. epidermidis* Y73 isolate as a probiotic

Since *S. epidermidis* is rarely used as a probiotic [19], it was of upmost necessity to take the antibiotic sensitivity profile of the isolate *S. epidermidis* Y73 into consideration, especially with the increasing prevalence of multidrug resistant (MDR) infections worldwide. In addition, this was followed as an insurance policy in case of any manifestation. In this study, the chosen antibiotics were selected following the same guideline as the physicians use in treating bacterial skin infections [23]. Disc diffusion method was used in this study to determine the antibiotic susceptibility, which is already used in several studies [24].

According to the results, *S. epidermidis* Y73 was sensitive for 13 tested antibiotics: Erythromycin, Cefotaxime, Cephalothin, Amikacin, Clindamycin, Ampicillin, Mxifloxacin, Trimethoprim, Trimethoprim-sulfamethoxazole, Azithromycin, Ciprofloxacin, Norfloxacin, and Ofloxacin. Whereas, it was only resistant to Ceftazidim and Colistin sulfate. Therefore, it can be said that *S. epidermidis* Y73 is multidrug sensitive, unlike the results obtained by Lazaris *et al* [25] who isolated a novel multi-resistance cfr plasmids in linezolid-resistant methicillin-resistant *S. epidermidis*. The other MRSE lacked cfr, but showed linezolid resistance-associated mutations. Taking into account that linezolid is often the drug of the last resort to treat infections caused by Gram-positive cocci; this strain could be very dangerous in case it has established an infection. Another study [26] reported a rifampicin resistant *S. epidermidis* isolate. Based on the previous data and with an overview concept, it is clear that *S. epidermidis* y73 is a safe isolate to be used in terms of antibiotic profile.

In addition, the optimal pH and temperature for *S. epidermidis* Y73 growth were investigated. In fact, a relatively wide range of pH was used to mimic the pH variation in human skin and to cover all the potential possibilities, since the normal pH value of the stratum corneum is 4.1–6. In addition, physiological gaps that include axillae, groin, toe, and anus exhibit pH values between 6.1 and 7.4. Therefore, using such a range is justified by the natural variety of the human skin pH [27]. As can be seen in Figure-3, a maximum *S. epidermidis* Y73 growth of 11.3 mg/ml was obtained at pH 7. However, the biomass was slightly decreased at pH 8 and 6 reaching to approximately 10 mg/ml.

On the other hand, several temperatures were investigated as a potential optimal condition and the results are shown in Figure-3. The optimal temperature for *S. epidermidis* Y73 growth was 37°C in which the biomass gained was 16.9 mg/ml. Based on these results, it can be said that this temperature is perfect since it is approximately equal to the human skin temperature. The above mentioned data of temperature, pH, and media were invested in further steps through the study to achieve the formula used in the application.

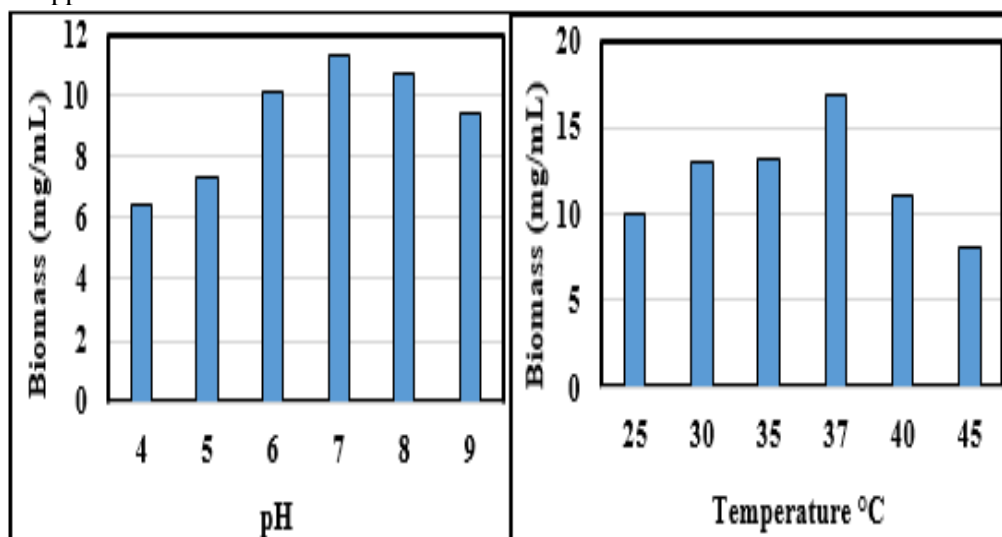


Figure 3-Effects of pH and temperature on *Staphylococcus epidermidis* Y73 grown in TSB.

S. epidermidis exists in healthy human skin as a commensal inhabitant and may become an important pathogen mainly if it has the ability of forming biofilms. Recently, increased resistance traits were suggested to be acquired in biofilm environments. Thus, both antibiotic resistant and biofilm formation are usually aligned. In a clinical study conducted by Sahal *et al.* [28], 65% of *S. epidermidis* were resistant to all b-lactam antibiotics (Penicillin, Oxacillin, Amoxicilin / Clavulonic

acid), and 60% of all *S. epidermidis* strains were found as multidrug resistant. However, the results of strong biofilm forming *S. epidermidis* showed that 80% of them were b-lactam resistant whereas 100% of them were multi drug resistant. As mentioned earlier in Table-1, *S. epidermidis* Y73 was non-biofilm producer based on the results obtained in the secondary screening. Consequently, by combining the data obtained through the study, *S. epidermidis* Y73, as a multidrug sensitive, non-biofilm forming, and strong bacteriocin producer isolated from normal healthy skin, is the elite candidate to be selected.

Probiotic application of *S. epidermidis* Y73

The use of living bacterial cells as probiotics was attended in several studies especially for bacteria like *Lactobacillus*, while for *S. epidermidis* only few attempts were performed. Cleland *et al.* [29] conducted a study in which *S. epidermidis* cells were introduced as cells without any immobilization to treat artificially induced rhinosinusitis infection with *S. aureus*. In that study, the ratio between the probiotic microorganism and the infection causing bacteria was 2 to 1; this is quite similar to the conclusion achieved in the current study. In addition, the use of *S. epidermidis* as a probiotic was fruitful in the mentioned study as it has eliminated *S. aureus* infection as well as prevented its ability to form biofilm in the site of colonization. On the other hand, Yang *et al* [19] also used *S. epidermidis* as a skin probiotic patch against *Cutibacterium acnes*, but in this scenario the cells were encapsulated with full immobilization. *S. epidermidis* mediated the fermentation of glycerol to produce antibacterial substances against *Cutibacterium* and the final result was a successful attempt to treat acne using that probiotic.

In this study and since the goal is to treat skin infection, finding a middle ground was attempted through introducing the probiotic cells to the infected area with partial immobilization on a cellulose paper pad. This was associated with making an advantage of the nature of *S. epidermidis* as non-motile bacteria so that the cells will be restricted to the edges of the pad, as shown in the Figure-4.

As mentioned earlier, 4 pad formulae were tested. The presence of petroleum jelly in both concentrations used in the formulae 2 and 4 acted as some sort of impediment to *S. epidermidis* Y73, which may be due to its thick consistency. Whereas, the use of only *S. epidermidis* Y73 cells in TSB was efficient and the probiotic cells swept the *S. aureus* 3. However, since the final design requires the probiotic cells to remain vital for a long period, glycerol was added to serve as another strength factor since *S. epidermidis* usually utilizes glycerol as a carbon source to produce succinic acid which has anti-bacterial properties [30]. This will certainly give the formula number 3 a very good multifactorial core structure, which led to its selection as the best candidate formula to prepare the pad containing *S. epidermidis* Y 73 cells.

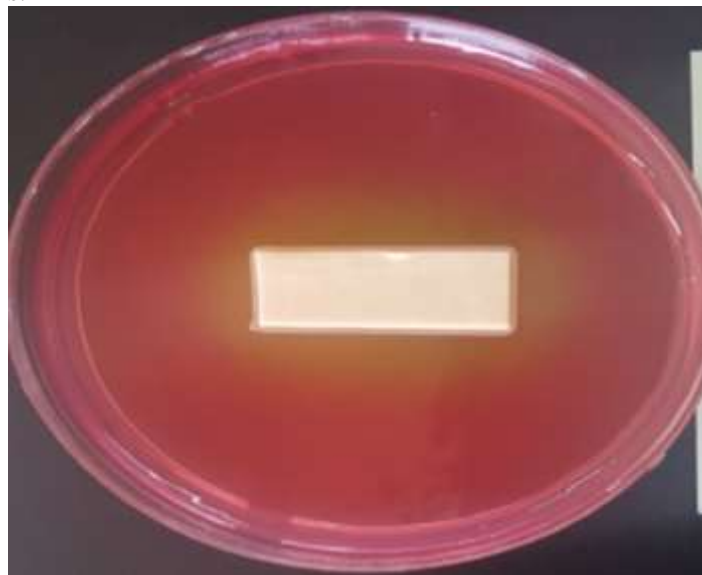


Figure 4-The pad containing *Staphylococcus epidermidis* Y73 cells on MSA.

Estimation of expiration date of the pad

Several expiration date estimation methods were proposed by Capen *et al.* [20], among which the original one inspired our work and was applied in the current study; a method in which the vitality of

cells was estimated and measured along a 20-week trial. The pads were all prepared at zero time and stored in sealed sterile tubes at 4°C; a single pad was extracted each week and placed on MSA to investigate the bacterial viability. The bacterial growth was represented by the yellow color around the pad as well as the bacterial growth at the edges. In addition, the purity of growth was examined and the same bases of diagnosis were followed. A slight reduction in the growth was noted after the 9th week, but in general the cells remained vital until the 20th week. It was found that *S. epidermidis* Y73 growth was restricted to the borderline of the pad. The appearance of the yellow color, which was due to the presence of glycerol in the pad formula, serves two purposes; (I) an indicator for the fermentation of carbon source that reflects the viability of *S. epidermidis* Y73 cells and (II) prolonging the shelf life of the pad.

In vivo application of *S. epidermidis* Y73 pad

The selected pad formula was used to treat artificially induced wounds on lab animals' skin. The wounds were infected with three clinical pathogenic isolates of *S. aureus*, *Kocuria rosea* and *P. aeruginosa*. In all cases, the same cut (approximately 2.5 cm) was conducted on the skin of the animals, and the excess blood was removed to avoid the direct contact between the inoculum (10⁴ cells per 100 microliter) and the immune product found in the blood.

. The three indicator species in this experiment were selected as common bacteria causing skin infections and the healing process was compared between the control and treatment groups.

Case 1: *Staphylococcus aureus*

S. aureus is responsible for most skin and soft tissue infections. Half of patients treated for primary skin infections experience recurrence within six months, despite sufficient antibiotic sensitivities and interventions to prevent infection [31]. *S. aureus* inhabits the skin in 20–30% of the population and induces 80–90% of all skin and soft tissue infections in humans worldwide [32,33,34]. The major infections fall into one of the following categories (I) Localized skin infections, (II) Deep, localized infections, and (III) Scalded skin syndrome.

Based on the results, it was clear that, with an overview perspective, the healing process with the presence of the *S. epidermidis* Y73 pad was faster, as shown in Figure-5. By the time of day 6, there was no trace to the wound in the treated animals, unlike the control. In addition, the pruritic rigid edges of the wound were noticed in the control, while in the presence of the pad the edges were smooth and the animal almost neglected the site of wound. Moreover, tenuous erythema was appeared in days 1 to 4 in the control, unlike the treated wound in which no redness was observed.

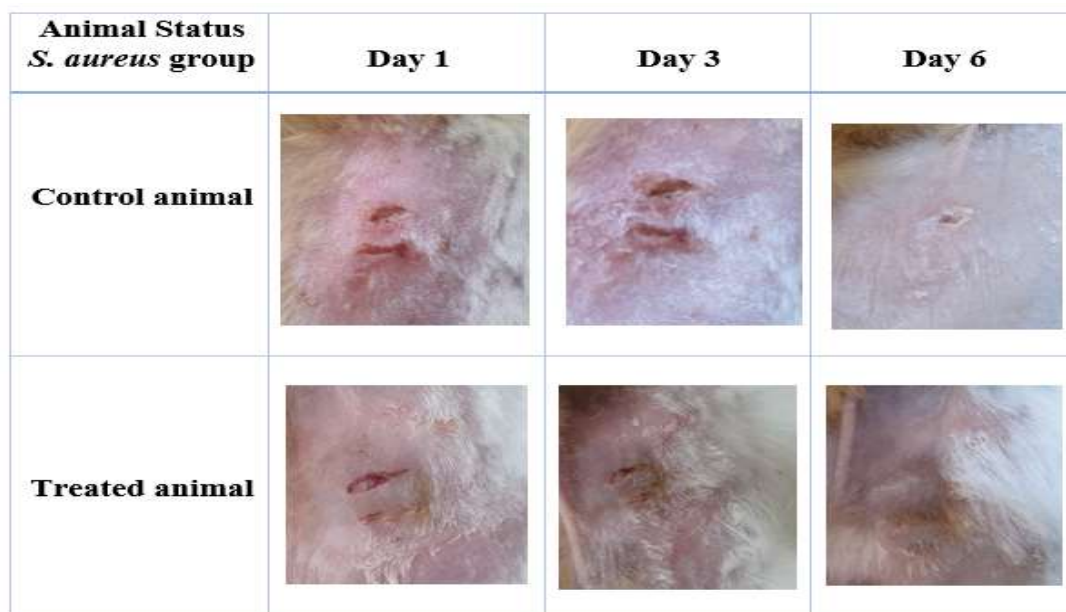


Figure 5- *In vivo* experiment for applying *Staphylococcus epidermidis* Y73 pad to treat a group of rabbits infected with *Staphylococcus aureus*

Case 2: *Kocuria rosea*

K. rosea was used in this study due to its newly rising titer as a potential pathogen worldwide [35]. Therefore, having sufficient data will aid to participate in dealing with any problem that may be caused by this Genus. The results of this experiment showed that the control rabbit infected with *K. rosea* suffered from high fever and severe diarrhea. Oncel and his colleagues recently reported a human infant with prolonged history of diarrhea with the blood culture growth containing *Kocuria* spp. [36]. Interestingly, the previous symptoms were not found in the treated animal. Moreover, it was obvious that the healing process was significantly improved in the treated wound Figure-6.

Kocuria spp has been reported to be normal human skin and oral cavity flora and is generally considered to be a laboratory pollutant, while overlooked when isolated in the clinical specimens which leads to undermining its pathogenic potential. Back in 1974, *kocuria* was first reported as a cause of urinary tract infection and was first named as *Micrococcus kristinae* [37]. Reports of *Kocuria* species infection gained prominence in the late twentieth century and displayed a growing trend, which indicates its pathogenic potential. *Kocuria*-isolated infections include infections of the urinary tract, cholecystitis, catheter-associated bacteremia, dacryocystitis, canaliculitis, keratitis, native valve endocarditis, peritonitis, mediastinitis, descending necrotizing, meningitis, and brain abscess [38, 39]. Based on the previously mentioned information, this bacterium has the actual potential to become a serious pathogen which should not be underestimated.



Figure 6- *In vivo* experiment for applying *Staphylococcus epidermidis* Y73 pad to treat a group of rabbits infected with *Kocuria rosea*.

Case 3: *Pseudomonas aeruginosa*

The case of this bacterium was the most promising in terms of wound healing. After day 6, there was almost no trace for the wound, as shown in Figure-7. Furthermore, the animal totally neglecting the wound, which is a sign of being non itchy. In addition, mild erythema was noticed in days 1 and 2 in the control, unlike the treated animal. Similar to *Kocuria rosea* case, the control suffered from fever and relatively decreased normal range of movement when compared to the other cases.

P. aeruginosa is also known to be a major skin opportunistic pathogen as it has the required characteristics such as biofilm formation and antibiotic resistance. Such infections are often

characterized by vigorous pathogen development at the infection site and increasing antibiotic resistance. The opportunistic pathogen *P. aeruginosa* is accountable for a wide variety of infections in immunocompromised hosts [40]. Among the most important of these infections are those localized to soft tissues, containing chronic and burn wounds [40, 41].



Figure 7-*In vivo* experiment for applying *Staphylococcus epidermidis* Y73 pad to treat a group of rabbits infected with *Pseudomonas. Aeruginosa*

Conclusions

Nowadays, there is a global orientation towards untraditional methods of treating bacterial infections. The main trigger to this wave of orientation is the rising incidence of multidrug resistant bacterial infections. One of the examples on these previously uncommon methods is the use of benzoyl peroxide (BPO) instead of antibiotics to treat acne, with the key difference is that BPO does not induce antibiotic resistance [42]. There is an increasing interest in using probiotics in both sides of doctors and end users. Thus, the results achieved in the current study, which proves the efficiency of using vital cells of the isolate *S. epidermidis* Y73 as a probiotic, may hopefully participate in completing the image about such topic.

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