Original Article

# The Effects of *Bacillus subtilis* Probiotic on Cutaneous Wound Healing in Rats

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Received: 20 July 2016; Accepted: 15 January, 2017

## Abstract

**Background:** The effect of several probiotics on diseases of the gastrointestinal tract, including gastric ulcers is well reported. However, there are limited numbers of studies assessing the impact of Bacillus subtilis (B. subtilis) probiotic on skin wound healing.

**Materials and Methods:** Fifty male rats were assigned to 3 different groups randomly. A 1.5 cm longitudinal full thickness incision was made in the back of each rat. Two groups, experimental 1 and 2, were treated by *B. subtilis* probiotics that were added to Eucerin. A control group was treated with Eucerin without any probiotic. Clinical changes and histopathological effects of *B. subtilis* probiotics on wound healing were evaluated every day and on 1, 3, 7, 14 and 21 days post-operation respectively.

**Results:** The percentage of wound healing in the experimental groups on day 7, when compared with the control group, was significant ($p \leq 0.05$). In the remaining days, there was also a progressed increase in wound healing, but there was no significant difference between control and experimental groups. The difference in the number of immune cells (neutrophils, macrophage and lymphocytes) in the experimental groups on different days of the study was statistically significant when compared to the control group.

**Conclusion:** The current studies demonstrate that *B. subtilis* probiotic is highly effective in enhancing skin ulcer healing. However, further studies would be necessary to elucidate the exact role of *B. subtilis* probiotic in would healing process.

**Keywords:** *Bacillus subtilis*, Probiotic, Cutaneous wound

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**Please cite this article as:** Shahsafi M. The Effects of *Bacillus subtilis* Probiotic on Cutaneous Wound Healing in Rats. Novel Biomed. 2017;5(1):43-7.

## Introduction

Skin as the first line of defense against invading microbes plays effective roles in maintaining human health[1]. Loss of the integrity or excision of the skin as a result of injury, illness, or surgery leads to major disturbances of this barrier function[1]. The ability of the skin to repair itself is remarkable, but in the severe injury, medical intervention is required, both to speed the wound closure and to protect the body from infection[2,3].

Several therapeutic agents are now widely used for wound healing. For example, previous studies have indicated that the application of epidermal growth factor and cytokines accelerates the rate of wound healing[4,7]. However, clinical results using these factors have been less than encouraging, and their potential roles in the chronic wound therapies remain to be established. Several authors have previously reported that certain probiotic bacteria, such as *Lactobacillus* spp., exhibit healing and antimicrobial activities[8-10]. The endorsed definition of probiotics is live microorganisms that, when administered in adequate amounts, confer a health benefit on the host[11]. Currently, the most well-studied probiotics are the lactic acid bacteria, particularly lactobacilli, and to a lesser extent, bifidobacteria. Other organisms considered for use as probiotics in humans include various *Bacteroides* spp., *Propionibacterium* spp., and *Bacillus* spp[12]. Recent reports have suggested that the healing and antimicrobial activities activity of *Lactobacillus* sp. probiotics involves production of exopolysaccharides, secreted compounds, as well as various antibiotics[13,14]. Probiotic *Bacillus subtilis* is also of interest because of its safety for use in humans. Till date, few studies have been done to assess the effect of these bacteria on cutaneous wound healing. Therefore, the aim of the present study was to determine the wound healing activity of probiotic strain *B. subtilis*.

## Methods

**Animal**: Fifty male Wistar rats weighting 150–200 g were housed under controlled conditions at a constant temperature (25C) and in 12-h-light/-dark cycle. Following a 14-day acclimation period, rats were randomized into experimental (treated either by lived probiotic or dead probiotic) and control groups. The Animal Experiment Ethics Committee of the University of Tehran approved this study.

**Induction of wounds:** First, the rats were anaesthetized with ketamine (150 mg/kg) /xylazine (750 mg/kg), and then a 1.5 cm2 full thickness open excision wound was made in the back of each rat. The wounds were not covered with dressing and the day of the surgical damage procedure was taken as day 0. After the wounding process, each rat was housed in a sterilized cage and given food and redistilled water in order to prevent bacterial infection.

**Administration of probiotic:** In order to prepare the ointment, $10^{10}$ CFU/ml bacteria that had been collected after a 48-h culture were added to Eucerin. The culture and Eucerin were mixed thoroughly until a uniform income produced and 1g of mixed sample applied to the wounds in the experimental groups. However, for the control group, 1g of Eucerin without any probiotic was used.

**Assessment of ulcer size:** The ulcer area and percentage ulcer healing at different times after treatments were assessed in rats. The length and width of wound (mm2) were measured in days 1, 3, 7, 14 and 21. The percentage healing was calculated as:
Percent healing = (Ulcer area in first day)-(Ulcer area in specific day)/(Ulcer area in first day)

**Histological examination:** Rats were killed on days 1, 3, 7, 14 and 21, by ether inhalation and tissues from the wound site, including the whole thickness of the skin and the surrounding skin of the individual animal was removed. The tissue samples were sectioned out and fixed overnight in 10% neutral buffered formalin, then, dehydrated gradually in ethanol and embedded in paraffin. Sections of 5 mm were obtained in an automated microtome. Ulcerated sections were stained with haematoxylin-eosin (H&E) for histological evaluation during healing.

**Statistical analysis:** Statistical analysis was performed using ANOVA test to determine significant differences between groups. Data were analyzed using Graph Pad software version 6.

## Results

**Microscopic analysis of wound healing:** H&E staining revealed different histological signs of healing among the different groups. As shown in Figure 1, wound healing on day 3 of treatment was not significant in either the control or experimental groups. Thereafter, there was a progressed increase in ulcer healing on day 7 in animals treated with dead probiotics (p= 0.03) as compared to untreated controls (Fig. 1). In the remaining days, there was also a progressed increase in wound healing, but there was

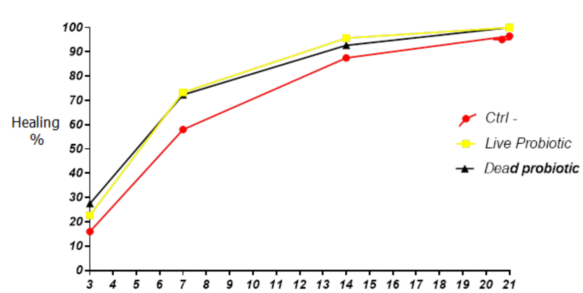


**Figure 1. The rate of wound healing in control, group treated with live probiotic (experimental 1) and group treated with dead probiotic (experimental 2) at different days.**

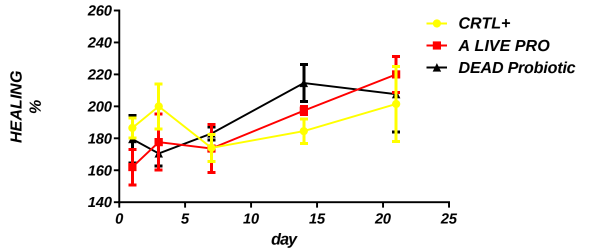


**Figure 2. Rates of weight gain in control, group treated with live probiotic (experimental 1) and group treated with dead probiotic (experimental 2) at different days.**

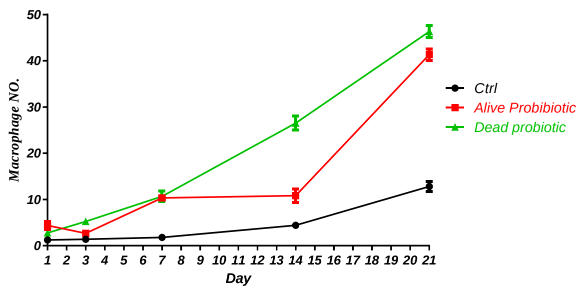


**Figure 4. The increased rate of macrophage production in control, group treated with live probiotic (experimental 1) and group treated with dead probiotic (experimental 2) at different days.**

no significant difference between control and experimental groups. The percent of healing in the control group was 16%, 58%, 87.5% and 96.5% on day 3, 7, 14 and 21, respectively. The same percentage was also seen in the experimental groups.

**Effect of probiotic treatment on weight of rats:** As shown in Figure 2, even though the weight was modestly increased in control and treated animals, it was not significantly different among the groups tested.

**Effect of probiotic treatment on neutrophil production:** Production of neutrophil was found to be elevated in the ulcerated tissues in the three groups of animals (Figure 3). Thereafter, the difference in the number of neutrophils in the experimental groups on the first, seventh and twenty first days of the study was statistically significant when compared to the control group.

**Effect of probiotic treatment on macrophage production:** On days 3 and 7 of the study, the number of macrophages in the experimental 1 group and in the experimental 2 group showed a significant

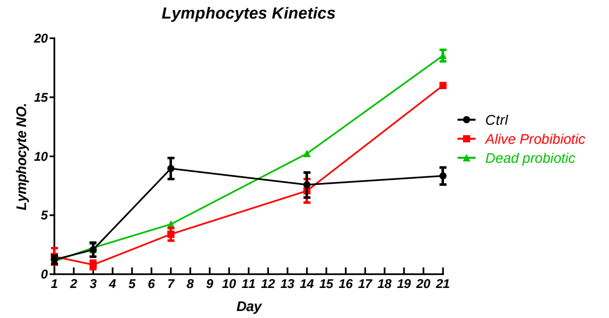


**Figure 5. The increased rate of lymphocyte production in control, group treated with live probiotic (experimental 1) and group treated with dead probiotic (experimental 2) at different days.**

**Figure 3. The rates of neutrophil reduction in control, group treated with live probiotic (experimental 1) and group treated with dead probiotic (experimental 2) at different days.**

increase in contrast to the control ($P<0.001$). Cell counting results also confirmed a significant increase in the number of macrophages on the fourteenth and twenty-first days ($P<0.001$) in the experimental groups than the control group (Figure 4).

**Effect of probiotic treatment on lymphocyte production:** As shown in Figure 5, there was a significant difference in the number of the lymphocyte production in the ulcerated tissues on days 7 and 21 in animals treated with live or dead probiotic as compared to animals treated with Eucerin only.

## Discussion

Wound healing is a dynamic process, which has three phases, including inflammation, tissue formation, and tissue remodeling that overlap in time[1]. A variety of medical substances can accelerate wound healing. Recently, the therapeutic role of probiotics is well reported in cases of ulcerative colitis, Crohn's disease and gastric ulcers[10,15,16]. However, there are limited numbers of studies assessing the impact of probiotic treatment on skin wound healing.

In the present study, we have observed a highly effective role of probiotic *B. subtilis* in promotion of healing of wound skin. Wound healing properties of other available probiotics themselves and their derived products have been previously described. For example, Rodrigues *et al.* treated wound in rats with kefir and increased the wound healing process[8]. Protective effects on the skin, connective tissues have been also reported by Zahedi *et al.* who reported that

*Lactobacillus* aid to healing in rats with damaged skins[17]. A similar effect due to a probiotic strain of *B. subtilis* is reported by our study. *B. subtilis* is a gram-positive, nonpathogenic, spore-forming organism normally found in the soil and the gastrointestinal tracts of some animals. The beneficial effects of *Bacillus* species on the wound healing in humans and animals are the basis of the rationale for their use as probiotics in the treatment of skin ulcers. These probiotics are available as pharmaceutical and food preparations in different countries in the world, even though little is understood about how these bacteria exert their therapeutic benefits[18,19]. Bacteria of this genus are able to produce specific substances such as bacteriocins, antibiotics and exopolysaccharide, that may would accelerate the wound healing process[12,20]. The production of immune cells, such as neutrophil, macrophage and lymphocytes was also found to be significantly higher in experimental groups as compared with control. These immune cells have been shown to play an important regulatory role in the wound healing and scar formation by initiating an early inflammatory process as well as several other immune responses[21,22].

# Conclusion

Our studies demonstrate that *B. subtilis* probiotic is highly effective in enhancing skin ulcer healing. However, further studies would be necessary to elucidate the exact role of *B. subtilis* probiotic in would healing process.

# Acknowledgment

The author would like to thanks Azad University of Arak for their financial supports of this paper.

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