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Positive Effects of Spray-Dried *Streptococcus thermophilus* on Healing of Second-Degree Burn Wounds in Wistar Rats

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ABSTRACT

Background and Objectives: Current medications used for the treatment of burn wounds have some side effects and disadvantages. Oral or topical administration of probiotic bacteria is thought to be useful for treatment of skin disorders. The aim of this study was to evaluate effects of spray-dried *Streptococcus thermophilus* on healing of second-degree burn wounds.

Methods: After induction of second-degree burn on back of 80 male Wistar rats, the animals were randomly allocated to negative control (no treatment), first vehicle control (treatment with Eucerin), second vehicle control (treatment with skim milk) and experimental (treatment with bacterial ointment) groups. Wound healing rate (percent) and histopathological parameters of wound samples were evaluated on post-burn days 1, 3, 7 and 14.

Results: On days 3 and 7, macroscopic results showed that the healing rate was significantly higher in the experimental group compared to the control groups. Histopathological analysis of wound samples showed increased fibroblastic migration, collagen formation and re-epithelialization in the wounds treated with probiotic bacteria compared with the wounds of control groups.

Conclusion: Our results indicate that the topical use of spray-dried *S. thermophilus* could be useful for the treatment of burn wounds.

Keywords: *Streptococcus thermophilus*; Probiotics; Burns; Wound Healing; Rats.

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INTRODUCTION

In spite of the fact that burns are preventable injuries (1), epidemiological studies indicate that the incidence of burns is still relatively high, especially in developing countries (2). Burn wounds are classified into first-, second- and third-degree injuries (3). Because the wound area is prone to infections, topical or systemic antibiotics are frequently used for burn management (4), which may be associated with undesirable side effects and antibiotic resistance (5, 6). Nowadays, probiotics are proposed as suitable alternatives to antibiotics for treatment of diseases (7). A probiotic is defined as a viable microorganism, especially bacteria, that can exert beneficial effects after being taken by the host (8). New research indicates that oral or topical use of bacterial probiotics may be effective in treatment of skin disorders (9-13). Previous studies show that local application of *Streptococcus thermophilus* on the skin may increase the skin's ceramide level in the epidermis, which may be useful in the treatment of atopic dermatitis (14). Moreover, it has been shown that the use of topical ceramide by itself can accelerate the wound healing process (15). Therefore, it may be concluded that the use of *S. thermophilus* on the wounds may increase the speed of wound healing.

One of the low-cost methods for long-term preservation of bacteria is spray-drying (16). Previous studies indicate that spray-dried bacteria including some lactic acid bacteria maintain their antagonistic activity against opportunistic bacteria that may infect the wounds, such as *Staphylococcus aureus* (17). In addition, spray-drying of lactic acid bacteria has no effect on their bacitracin production potential (17, 18). Studies have investigated the effects of topical use of free cultures of probiotic bacteria on wound healing (19, 20), but no study has yet evaluated the effects of topical spray-dried lactic acid bacteria on this process. Thus, the purpose of the present research was to evaluate the effects of spray-dried *S. thermophilus* on the wound healing process in a rat model of second-degree burn wound.

MATERIALS AND METHODS

Male Wistar rats were obtained from the Pasture Institute of Tehran, Iran. Before starting the experiments, the rats were kept In a standard room, under 12/12 light-dark cycle and temperature of about 22 °C. The animals had ad libitum access to food and water at all times. All experimental procedures were approved by the ethics committee of University of Maragheh (approval code: 96083155). A standard method used by Pereira et al. was adopted for induction of second-degree burn wounds (21). Briefly, the rats received intraperitoneal anesthetic mixture of 80 mg/kg ketamine and 10 mg/kg xylazine (both purchased from Alfasan Company, Netherlands).

Hair of back skin, near the forelimbs, was removed with a clipper. Then, the hairless area was antisepticised and base of a solid aluminum rod (diameter=10 mm) pre-exposed to temperature of 96 °C was placed on the area for 15 seconds. Next, the rats were placed in separate cages and received buprenorphine, which has relatively long analgesic effects (22).

Spray-dried probiotic *S. thermophiles* (ATCC 19258) was obtained from Zisttakhmir Corporation (Tehran, Iran) in a powder containing $10^{10} - 10^{11}$ CFU of the bacteria per gram sample.

The experiments were conducted in 1-, 3-, 7- and 14-day periods. On the day of burn induction (day 0), the rats were randomly divided into 16 groups of 5. Four groups (subgroups 1, 2, 3, 4) were assigned as negative controls and received no treatment; they were euthanized after 1, 3, 7 and 14 days post-burn, respectively.

Four groups (subgroups 5, 6, 7, 8) were assigned to first vehicle controls and received Eucerin every day for 1, 3, 7 and 14 days, respectively. Four groups (subgroups 9, 10, 11, 12) were assigned to the second vehicle controls and received daily skim milk for the same period as the first vehicle controls did. The remaining four groups of rats (the experimental groups) received the bacterial powder in Eucerin every day just like the time schedule of vehicle controls treatment.

Assessment of the wound healing process was made by calculating the wound healing percentage on days 1, 3, 7 and 14 of the experiments and by histopathological analysis of the wound areas using wound samples on the same days.

The following formula was used to calculate the healing percentage:

$$Wound\ healing\ percentage\ on\ day\ x = \left(1 - \frac{wound\ area\ on\ day\ x}{wound\ area\ on\ day\ 1}\right) \times 100$$

For evaluation of histological parameters as measures of wound healing, biopsy specimens of the wound tissues collected on days 1, 3, 7 and 14 were fixed, processed, embedded in paraffin blocks and sectioned. The tissue sections (5 μm thick) were stained with hematoxylin and eosin. The parameters analyzed for the wound healing assessment included inflammatory response, fibroblast migration, formation and orientation of collagen fibers and re-epithelialization. A histologist, blind to the conditions of the experiments, scored the parameters by averaging the intensity of the above parameters in five visual field of each slide: (–) absence, (+) mild presence, (++) moderate presence and (+++) strong presence (21).

SPSS software (version 19) was used for statistical analysis of quantitative data. Factorial ANOVA was used for determining possible interactions between time and treatments on the healing percentage on different days. LSD post hoc test was done for significant results. Data were expressed as mean ± SEM.

RESULTS

On the third day, the wound healing rate (%) of the experimental group was significantly higher than that of the control groups except for the second vehicle control group. On day 7, the wound healing rate in the experimental group was significantly higher than in all control groups. On day 14, the wound healing rate was significantly greater in the experimental group compared to both vehicle groups (Figure 1).

Microscopic evaluation of the wound area on the first post-burn day showed the disruption of epidermis, presence of cavities in damaged tissues and denaturation of collagen fibers. Altogether, on day one, the structural damages observed in all groups were almost identical (Figure 2). On the third day, the inflammatory response in the experimental groups reduced compared to both vehicles and negative control groups (Figure 3 and Table 1). On the next day, the greatest reduction in the inflammatory response was observed in the experimental group (Figures 4 and 5). On post-burn days 7 and 14, the experimental group had the highest rate of collagen fiber formation, and the fiber had more regular patterns than other groups (Figures 4 and 5). Finally, the rate of re-epithelialization in the experimental group was higher than in the control groups on day 14 (Figure 5).

Table 1- Histopathological parameters of wound areas on post-burn days 1, 3, 7 and 14.

Group	*Day*	*Inflammatory response*	*Fibroblast migration*	*Collagen formation*	*Epithelialization*
Negative control	**1st**	++	-	-	-
	3rd	+++	+	-	+
	7th	++	++	+	+
	14th	+	++	++	++
First vehicle control	**1st**	++	-	-	-
	3rd	+++	+	-	+
	7th	++	++	+	+
	14th	++	++	++	++
Second vehicle control	**1st**	++	-	-	-
	3rd	+++	+	-	+
	7th	++	++	+	+
	14th	++	++	++	++
Experimental	**1st**	++	-	-	-
	3rd	++	+	-	+
	7th	+	+++	++	++
	14th	+	+	+++	+++

Figure 1- Wound healing rate (%) in the control and experimental groups on days 1, 3, 7 and 14.

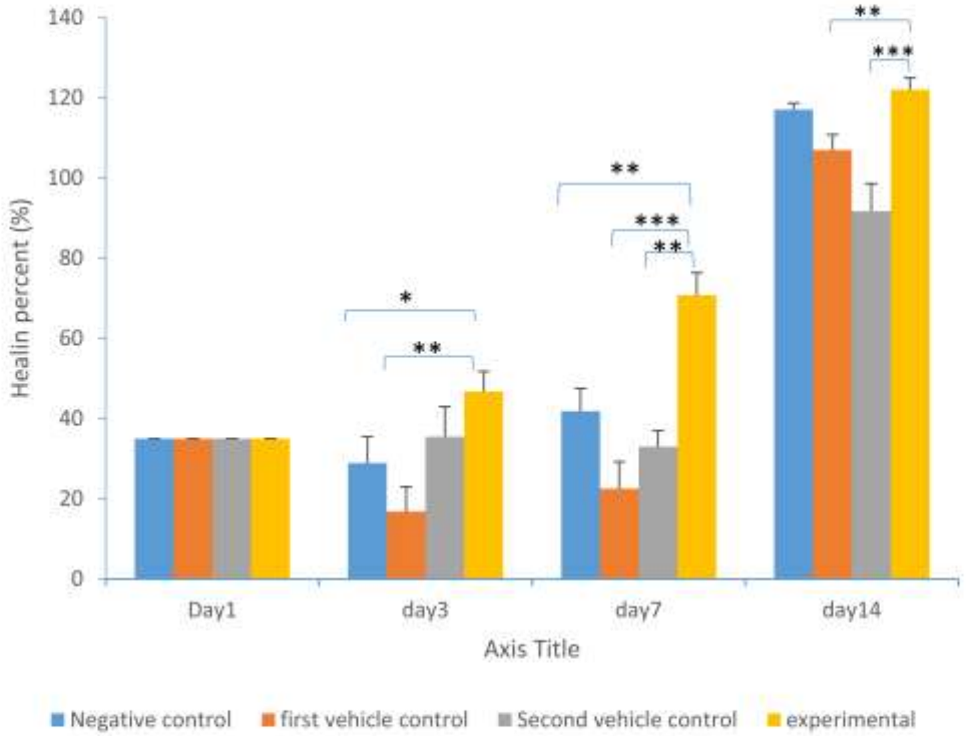


Figure 2- Microscopic images of the wound tissue samples on the first day of the experiments. (a): negative control, (b): first vehicle control, (c): second vehicle control and (d) the experimental groups. The arrowheads show the disruption of epidermis by heat. The arrows show thick collagen fibers with distorted structure caused by the high temperature. The star represents the cavities created in the damaged tissues. The images were captured at 400x magnification.

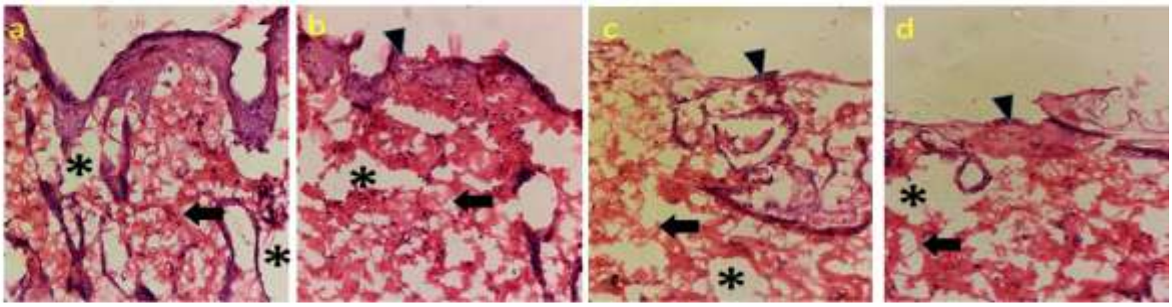


Figure 3- Images of tissue sections of the wound samples on the third day. There was a wide infiltration of inflammatory cells to the wound area. The density of inflammatory cells in the experimental group (d) was lower than in the control groups (a, b and c). Yellow arrows show inflammatory cells. The images were captured at 400x magnification.

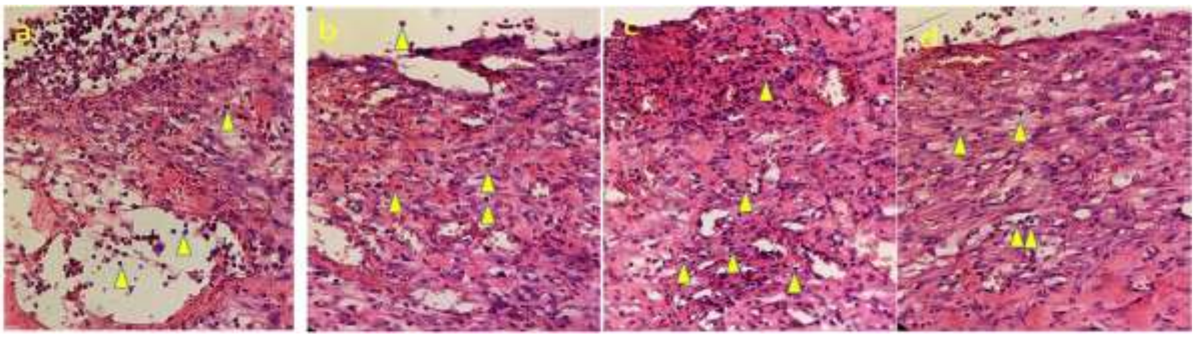


Figure 4- Images of tissue sections of the wound samples on day 7. The density of inflammatory cells (yellow arrowheads) in the tissue sections of the experimental group (d) was still lower than that of the control groups (a, b and c). In addition, in the experimental groups, collagen fibers had a more regular structure and arrangement compared with the control groups. More cavities can be observed in the dermis layer of samples from the negative and positive control groups (a, b, and c) compared to the experimental group. Two-way arrows show the formation of epidermis in all groups. Blue arrows and black arrows represent fibroblasts and collagen fibers, respectively. The images were captured at 400x magnification

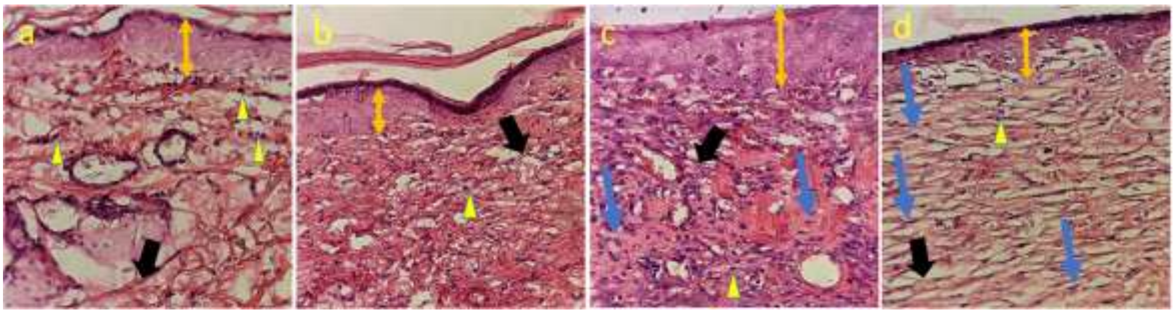
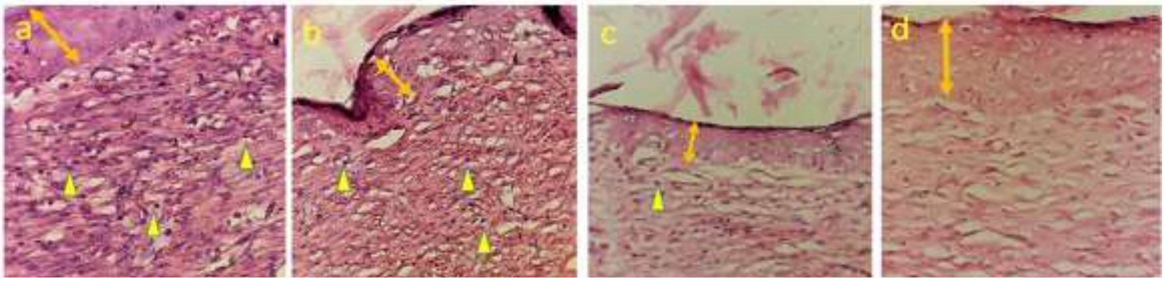


Figure 5- Images of tissue sections of the wound samples on day 14. The epidermis (bidirectional arrows) in the experimental group (d) was thicker than in other groups (a, b and c). In the experimental group, inflammatory cells with circular and dens colored nuclei were less scattered. In this group, the collagen fibers were also thicker and had a more regular pattern compared to other groups. The images were captured at 400x magnification



DISCUSSION

In the second- and third-degree burns, the healing process involves inflammatory response, proliferation and remodeling phases (23). Our experimental results showed that the inflammatory response in the wounds treated with *S. thermophilus* was less intense compared to the control groups, especially the vehicle controls. Previous research indicates that the bacteria may have some anti-inflammatory effects (24-26). Moreover, the antibacterial properties of *S. thermophilus* against some opportunistic bacteria that may cause wound infection have been demonstrated (27). Infection of the wounds by fewer pathogens would cause a less intense inflammatory response, a situation observed in our experimental groups. If the inflammatory phase ends sooner, the proliferative phase will begin in a shorter time; therefore, the whole process of wound healing takes less time. In line with the microscopic results, on day 3, the macroscopic results showed that the wound healing rate was significantly higher in the experimental group compared to the control groups. In the present study, the highest wound healing rate was observed in the experimental group on the seventh day of the experiments. On the same day, the rate of processes that occur in the proliferation phase of wound healing including fibroblasts migration, formation of collagen and re-epithelialization was also highest in the experimental groups. On day 14, the collagen fibers arrangement in the experimental group was more regular than in other groups and had a nearly reticular pattern, which is indicative of normal skin in rodents (28).

On day 14, the rate of wound healing in the experimental group was higher than both vehicle controls but not the negative control group. The vehicle controls received moisturizing ointment during the study period, which makes the wound area more suitable for the growth of opportunistic bacteria, such as *Pseudomonas aeruginosa* (29, 30). However, these effects were eliminated in the experimental groups due to the antibacterial properties of *S. thermophilus* (31).

Another reason for the positive effects of *S. thermophilus* on the wound healing process is the production of ceramide (14), which can have healing effects on the wounds (15). Altogether, our results indicate the beneficial effects of spray-dried *S. thermophilus* on burn wound healing process. These results are in line with the results of other studies. For example, Peral et al. showed that topical use of *Lactobacillus plantarum* had positive effects on healing of second- and third-degree burn wounds (19). Moreover, administration of kefir grains that contain a mixture of probiotic bacteria on wounds can accelerate the healing process (20, 32).

S. thermophilus is generally regarded as safe since it lacks virulence-related genes (33, 34) and has been widely used in the dairy industry. Therefore, the topical use of these bacteria on skin may be also safe. Furthermore, spray drying is an affordable and simple method for preservation of viable bacteria.

The skin of rat is different from that of humans both in terms of structure and function (35-38), and the healing process of a skin wound in the two species differs in some aspects (39). Therefore, more studies on animals with a more similar skin to that of humans or on volunteer patients are essential to confirm the positive effects of *S. thermophilus* on burn wound healing.

CONCLUSION

Our results indicate that the topical use of spray-dried S. *thermophilus* could be useful for the treatment of burn wounds.

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CONFLICT OF INTEREST

We have no conflict of interest to declare.

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