

## RESEARCH ARTICLE

# *Lactobacillus rhamnosus* GG and *Bifidobacterium animalis* subsp. *lactis* BB-12 promote infected wound healing via regulation of the wound microenvironment

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## Abstract

Infected wounds can result in complex clinical complications and delayed healing, presenting a significant global public health challenge. This study explored the effects of topical application of two probiotics, *Lactobacillus rhamnosus* GG (LGG) and *Bifidobacterium animalis* subsp. *lactis* BB-12, on the microenvironment of infected wounds and their impact on wound healing. LGG and BB-12 were applied separately and topically on the *Staphylococcus aureus* (*S. aureus*)-infected skin wounds of the rat model on a daily basis. Both probiotics significantly accelerated wound healing, demonstrated by enhanced granulation tissue formation and increased collagen deposition, with BB-12 showing superior efficacy. LGG and BB-12 both effectively inhibited neutrophil infiltration and decreased the expression of pro-inflammatory cytokines tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ) and interleukin-6 (IL-6). Notably, BB-12 markedly reduced IL-6 levels, while LGG significantly lowered TNF- $\alpha$ , transforming growth factor- $\beta$  (TGF- $\beta$ ) and vascular endothelial growth factor (VEGF). Additionally, both probiotics promoted macrophage polarization towards the anti-inflammatory M2 phenotype. Microbiota analysis revealed that LGG and BB-12 significantly decreased the abundance of pathogenic bacteria (e.g. *Staphylococcus* and *Proteus*) and increased the proportion of beneficial bacteria (e.g. *Corynebacterium*). Particularly, BB-12 was more effective in reducing *Staphylococcus* abundance, whereas LGG excelled in promoting *Corynebacterium* growth. These findings suggest the ability of LGG and BB-12 to modulate the wound microenvironment, enhance wound healing and provide valuable insights for the management of infected wounds.

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## INTRODUCTION

The wound microenvironment refers to the complex biological and biochemical environment within the wound, including cells, extracellular matrix, microbiota and wound pH (Wang, Qi, et al., 2022). Once the wound microenvironment is imbalanced, the wound is susceptible to bacterial infection, significantly hindering the healing process (Dhall et al., 2019; Mo et al., 2023). This imbalance often manifests itself as increased abundance of pathogenic microorganisms, changes in pH and impaired immune cell function, which weakens the body's defences against infection, delaying the healing processes. Therefore, regulating and restoring the balance of the wound microenvironment is essential to control wound infection (Zhang et al., 2022).

Currently, clinical treatments for infected wounds mainly include debridement, intravenous infusion, oral antibiotics and the application of antibacterial dressings (Han et al., 2024; Reigadas et al., 2021). Although these therapeutic measures aim to optimize the wound microenvironment, they are often accompanied by problems such as pain, secondary damage and bacterial resistance (Chowdhury & Findlay, 2023; Salisbury et al., 2018). Therefore, there is an increasing need to develop new safe and effective wound care technologies to promote wound healing.

Recent studies have further revealed the important role of skin microbiota in wound healing. The skin microbiota not only protects the skin from pathogens but also participates in the regulation of the immune system, thereby affecting the wound-healing process (Constantinides et al., 2019). For example, Baquer et al. (2023) found that skin microbiota can stimulate T cells to secrete IFN- $\gamma$  while inhibiting the expression of pro-inflammatory chemokines IL-8, CXCL1 and MCP-1, thereby reducing inflammatory responses. Scharschmidt et al. (2017) revealed the critical role of the skin microbiota in promoting the enrichment and localization of normal immune cells in the skin, while Enamorado et al. (2023) demonstrated that the skin microbiota can promote the repair and regeneration of sensory neurons. In addition, Wang et al. (2021) found that an imbalance in skin microbiota can inhibit the regenerative ability of skin and hair follicles. These findings indicate the critical role of the microbiota in the homeostasis of the wound microenvironment.

In this context, probiotics, as supplements to the commensal microbiota, may become a potential strategy to modulate the wound microenvironment by providing a protective barrier and modulating immune responses. Studies have attempted to explore the role of probiotics in wound healing. Ong et al. (2020) developed an ointment containing 10% protein fraction of *Lactobacillus plantarum* and applied it to wounds. The results showed that *L. plantarum* can inhibit the growth of *Staphylococcus aureus* (*S. aureus*), and enhance the expression of cytokines and chemokines, thereby

promoting the migration of keratinocytes and wound healing. Although topical probiotics show significant potential in wound care, results from existing studies show some heterogeneity due to differences in strain selection, dose, concentration, duration and administration method (Knackstedt et al., 2020). For example, Partlow et al. (2016) found that topical application of *Saccharomyces boulardii* did not accelerate wound healing or significantly alter the wound microbiota. Nevertheless, the diversity of these studies reflects the breadth of exploration in probiotic applications rather than methodological shortcomings.

To further advance this field, more targeted and systematic research is urgently needed, especially in clarifying the mechanisms of action of specific probiotic strains in specific wound environments. To address these issues, this study thoroughly investigates the specific roles of *Lactobacillus rhamnosus* GG (LGG) and *Bifidobacterium animalis* subsp. *lactis* BB-12 in modulating the wound microenvironment during the healing of infected wounds. For the first time, this study systematically compares the effects of these two probiotics on a rat skin infection model regarding their ability to reduce the pathogen load, modulate inflammation, reshape the wound microbiota and accelerate wound healing. Through multi-omics approaches, this study also uncovers the mechanism by which probiotics modulate the microbiota to enhance wound healing, providing a theoretical basis for developing new wound treatment strategies. The corresponding findings suggest that probiotics hold significant potential as alternatives or adjuncts for infected wound treatment, providing theoretical and experimental references for future research and clinical applications in this field.

## EXPERIMENTAL PROCEDURES

### Materials

LGG (ATCC 53103) and BB-12 (ATCC 50675) were purchased from Chr. Hansen Holding A/S, Hørsholm, Denmark, and cultured in de Man, Rogosa and Sharpe (MRS) agar and TOS propionate agar respectively. *Staphylococcus aureus* (ATCC 6538) and *Escherichia coli* (ATCC 23815) were purchased from Shanghai Luwei Technology Co., Ltd., and cultured on nutrient agar. De Man, Rogosa and Sharpe (MRS), tomato Juice (TOS) propionate agar and nutrients were purchased from Qingdao Hi-Tech Industrial Park, Haibo Biotechnology Co., Ltd. (Qingdao, China).

### Preparation of live LGG suspension

Live LGG was grown on MRS agar at 37°C for 48 h. From the agar, colonies were picked and inoculated

in MRS broth and cultured until the logarithmic growth phase was reached. The culture was stopped when the population was approximately  $10^9$  Colony-forming unit (CFU)/mL to obtain the mixture of live LGG suspension with its metabolites. The preparation scheme is summarized in Figure 1A.

### Preparation of live BB-12 suspension

Live BB-12 was grown on TOS agar at 37°C for 48 h. From the agar, colonies were picked and inoculated in tryptone-yeast (TPY) broth and cultured until the logarithmic growth phase was reached. The culture was stopped when the population was approximately  $10^9$  CFU/mL to obtain the mixture of live BB-12 suspension with its metabolites. The precreation scheme has been summarized in Figure 1B.

### In vivo experiments on infected wounds in rats

Animal experiments were approved by the Animal Ethics Committee of Sichuan University (Animal Ethics Filing Number: 20230515003) and conducted in accordance with the guidelines of the China Research Institute Animal Use Committee. Sprague Dawley (SD) male rats (8 weeks,  $220 \pm 10$  g) were purchased from Chongqing Enswell Biotechnology Co., Ltd. Anaesthesia was induced using isoflurane gas at an induction concentration of 3%–4% and a maintenance concentration of 2%–2.5%. As described earlier (Lu et al., 2022; Yang et al., 2023), a full-thickness skin defect wound with a diameter of 10 mm was established on the back of rats, and then 100  $\mu$ L *S. aureus* bacterial suspension ( $10^8$  CFU/mL) was applied to the wound and covered for 24 h. The 30 rats were randomly divided into three groups: control (10 rats), LGG (10 rats) and BB-12 (10 rats). Briefly, 500  $\mu$ L of LGG or BB-12 suspension ( $10^9$  CFU/mL) was applied to the wound,

whereas the control group did not receive any intervention. The weight, wound pH and wound healing status of the rats were observed and recorded daily, and the wound area was measured using ImageJ software according to the following equation:

$$\text{Wound area (\%)} = S_n / S_0 \times 100 \%$$

where  $S_0$  and  $S_n$  are the wound areas on days 0 and n respectively. The wound area has been represented as mean  $\pm$  SD.

### Blood sample analysis

Blood (0.5 mL) was collected from the tail vein of the rats before the intervention (before and after modelling) and 3 days after the intervention. Blood analysis was performed using a fully automatic veterinary blood cell analyser (Shenzhen Mindray Bio-Medical Electronics Co. BC-5000 Vet).

### Analysis of wound histology and immunology

Wound skin tissue was stained with H&E and Masson, and immunohistochemical studies were performed (IL-6, TNF- $\alpha$ , VEGF and TGF- $\beta$ ). The primary antibody for TNF- $\alpha$  was diluted 1:200 (BA0131, Boster Bio), VEGF was diluted 1:200 (bs-1313R, Bioss), IL-6 was diluted 1:200 (GB11117, Servicebio) and TGF- $\beta$  was diluted 1:200 (GB11117, Servicebio). Images of the stained sections were taken using the 3DHISTECH (Hungary) CaseViewer2.4 software and the data image analysis system Indica Labs (USA) Halo 101-WL-HALO-1. The wound tissue was immunofluorescent stained (CD206 and CD86) and observed under a fluorescence microscope (Eclipse E100, Nikon). The number of cells expressing CD86 and CD206 was analysed using ImageJ software.

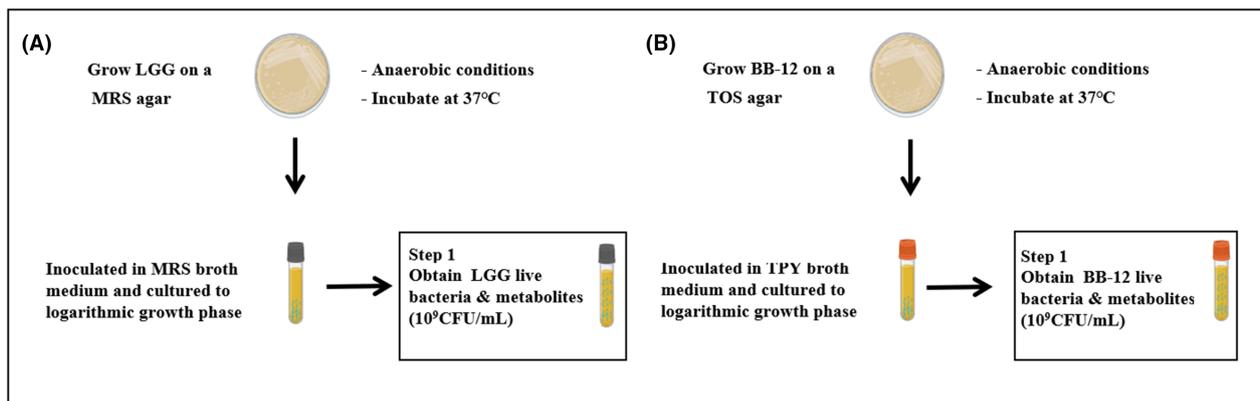


FIGURE 1 Preparation method for (A) live LGG and (B) live BB-12 suspensions.

## Analysis of wound microorganisms

Wound swab samples were collected using sterile swabs and cryovials and stored in a refrigerator set to  $-80^{\circ}\text{C}$ . A Tiangen DNA kit (Tiangen Science and Technology, Beijing, China) was used to extract the DNA from the wound tissue. 16SrRNA gene fragments were amplified with universal primers (forward primer, V3-338F5'-ACTCCTACGGGAGGCAGCAG-3'; reverse primer, V4-806R 5'-GGACTACHVGGGTWTCTAAT-3') (Ren et al., 2017). PCRs were conducted as 25- $\mu\text{L}$  reactions comprising 12.5  $\mu\text{L}$  of the Phusion® Hot Start Flex 2X Master Mix (New England Biolabs Inc., Ipswich, MA, USA), 2.5  $\mu\text{L}$  of the forward and reverse primers and approximately 50 ng of the template DNA (Cheng et al., 2018).

## Statistical analyses

Statistical analyses were performed using Origin software. All data were presented as the mean  $\pm$  SD. One-way analysis of variance (ANOVA) was used to determine significant differences between means at a significance level of  $p < 0.05$ . Tukey's test was used for multiple comparisons between the means.  $p$ -values were calculated to indicate the significance levels, presented as  $*p < 0.05$ ,  $**p < 0.01$  and  $***p < 0.001$ .

All experimental data were based on samples from 10 rats per group. For histological and immunofluorescence staining results, the average values from three different sections per sample were used for statistical analysis. The 16S rRNA-sequencing data were analysed using multiple comparison tests to assess differences in microbial communities between the experimental groups.

## RESULTS

### Effect of LGG and BB-12 on wound healing

LGG and BB-12 showed significant inhibitory effects against *S. aureus* (Figure S1 and Table S1), which is one of the most common pathogenic bacteria (Linz et al., 2023). Herein, an *S. aureus*-infected wound rat model was established as described earlier (Han et al., 2024) to evaluate the functions of LGG and BB-12 in vivo. The modelling process and intervention method are shown in Figure 2A. Topical treatment with LGG and BB-12 was performed on the wound after successful creation of the model. A representative image of the wound region in each group is shown Figure 2B. Accelerated wound closure was observed in the LGG and BB-12 groups compared to the control

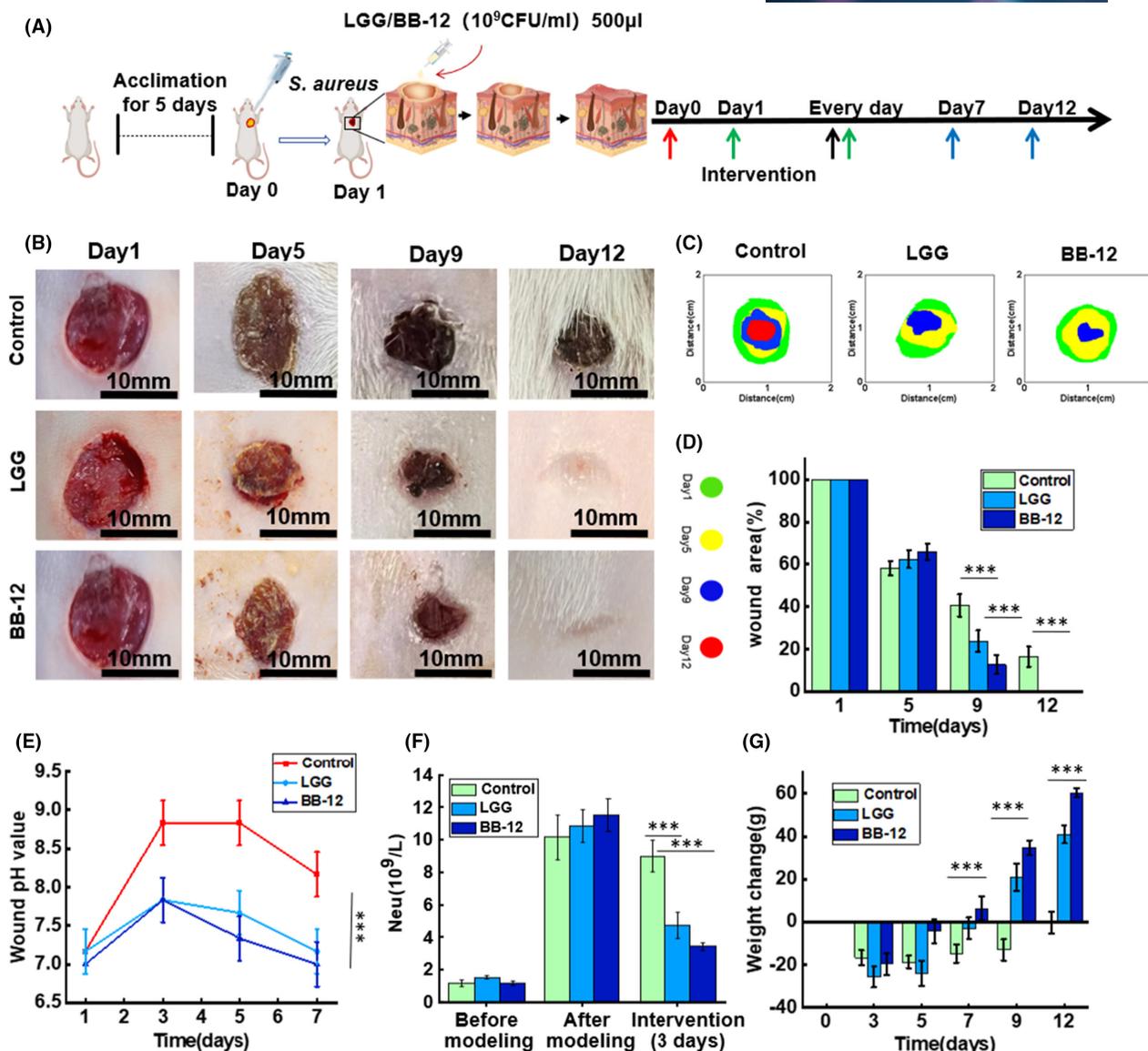
group (Figure 2C). In particular, the wound-healing rates of the LGG and BB-12 groups were 76.21% and 87.25%, respectively, after 9 days of intervention, which were significantly higher than that of the control group (59.43%). Notably, the wounds in the LGG and BB-12 groups completely healed on day 12, whereas the wounds in the control group remained unhealed on day 12 (Figure 2D).

Variations in the wound pH during the healing process are shown in Figure 2E. During the first 3 days, the pH of the wound increased, possibly owing to bacterial infection. From the 4th day, the pH gradually decreased to normal levels. Particularly, on the 3rd day, the pH of the wound in the LGG and BB-12 groups were  $7.83 \pm 0.28$  and  $7.83 \pm 0.36$ , respectively, which were lower than that in the control group ( $8.83 \pm 0.28$ ). Moreover, on the 7th day, the wound pH in the LGG and BB-12 groups was significantly lower than that in the control group ( $p < 0.001$ ). These results suggest that treatment with LGG and BB-12 can regulate the micro-environment of infected wounds.

### Wound histological analysis

Haematological analysis revealed that the neutrophil levels in each group increased beyond the normal range ( $0.35\text{--}6.30 \times 10^9/\text{L}$ ). After intervention (3 days), the neutrophil level in the LGG and BB-12 groups was  $4.78 \pm 0.83 \times 10^9/\text{L}$  and  $3.46 \pm 0.21 \times 10^9/\text{L}$  respectively. In contrast, neutrophil level in the control group was  $9.01 \pm 0.98 \times 10^9/\text{L}$ , which is beyond the normal range (Figure 2F). Additionally, weight changes in the rats during the healing process were recorded and are summarized in Figure 2G. Over the first 7 days, the weight of the rats was lower than its initial value owing to infection. After 7 days, it was found that the weight of the rats in LGG and BB-12 groups increased rapidly, reaching  $9.55 \pm 7.2\text{ g/day}$  and  $10.3 \pm 4.1\text{ g/day}$ , which were significantly higher than that of the control group with  $-0.33 \pm 5.32\text{ g/day}$  ( $p < 0.05$ ), suggesting that the infection and inflammation in the rats of the LGG and BB-12 groups were effectively controlled.

Skin defects are primarily repaired by the in situ growth of granulation tissue (Castaño et al., 2018; Zhong et al., 2022). As shown in Figure 3(A<sub>1</sub>), more granulation was observed in the LGG and BB-12 groups than in the control group. Furthermore, the granulation tissue in LGG ( $0.62 \pm 0.06\text{ mm}$ ) and BB-12 groups ( $0.82 \pm 0.05\text{ mm}$ ) was much thicker than in the control group ( $0.45 \pm 0.04\text{ mm}$ ) (Figure 3(A<sub>2</sub>)). Additionally, a higher amount of collagen deposition was observed in LGG ( $62.4 \pm 1.57\%$ ) and BB-12 ( $68.75 \pm 1.63\%$ ) groups than in the control group ( $23.4 \pm 1.86\%$ ) (Figure 3(B<sub>1</sub>), (B<sub>2</sub>)). Furthermore, the LGG and BB-12 groups showed almost completely epithelialization (red arrows), accompanied by the



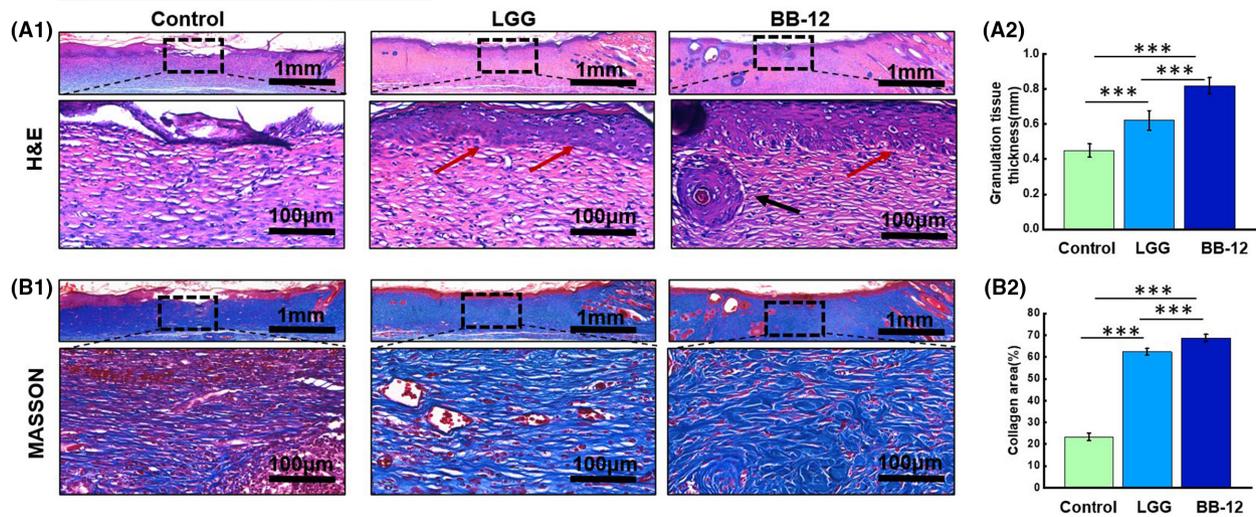
**FIGURE 2** (A) Animal experiment scheme. (B) Photos of wounds at various time points in each group over 12 days. (C) Unhealed wound areas on days 1, 5, 9 and 12. (D) Quantitative analysis of wound area at different time points in each group. (E) Wound pH on days 1, 3, 5 and 7. (F) Neutrophil levels before and after creation of the infected wound model, and 3 days of intervention. (G) Weight changes on days 3, 5, 7, 9 and 12 in each group.

appearance of hair follicle structures (black arrows). In contrast, no intact epithelial tissues were observed in the control group.

## Expression of immune cells and inflammatory factors

TNF- $\alpha$  and IL-6 are pro-inflammatory cytokines that play pivotal roles in the immune response and are often used as markers of inflammation and infection. Herein, the relative levels of TNF- $\alpha$  and IL-6 in each group were assessed by immunohistochemical analysis and the results are illustrated in Figure 4A. Compared with the control group, a statistically significant reduction in the expression of IL-6 and

TNF- $\alpha$  was observed in both LGG and BB-12 groups ( $p < 0.001$ ). Additionally, growth factors including VEGF and TGF- $\beta$  are crucial in the regulation of wound-healing processes. Immunohistochemistry results and statistical data are shown in Figure 4B, which revealed significantly lower expression levels of TGF- $\beta$  in LGG and BB-12 groups than in the control group on day 12 ( $p < 0.001$ ). Similarly, VEGF expression in LGG and BB-12 groups was significantly lower than that in the control group ( $p < 0.001$ ). Macrophage distribution and quantity within the wound were assessed using M1 (CD86, red) and M2 (CD206, green) phenotypic markers. Active expression of M1 macrophages can aggravate inflammatory responses. As shown in Figure 4C,D, cells positively stained for CD86 were fewer in LGG and BB-12 groups than in



**FIGURE 3** (A<sub>1</sub>) H&E staining images. (A<sub>2</sub>) Granulation tissue thickness. (B<sub>1</sub>) Masson's trichrome staining images. (B<sub>2</sub>) Collagen deposition in the wounds of each group on day 12.

the control group. Concurrently, a consistent upregulation of CD206-positive cells was observed in the LGG and BB-12 groups compared with the control group. These results indicate that topical application of LGG and BB-12 leads to a shift in macrophages towards a less inflammatory and more reparative phenotype.

## Wound microbial analysis

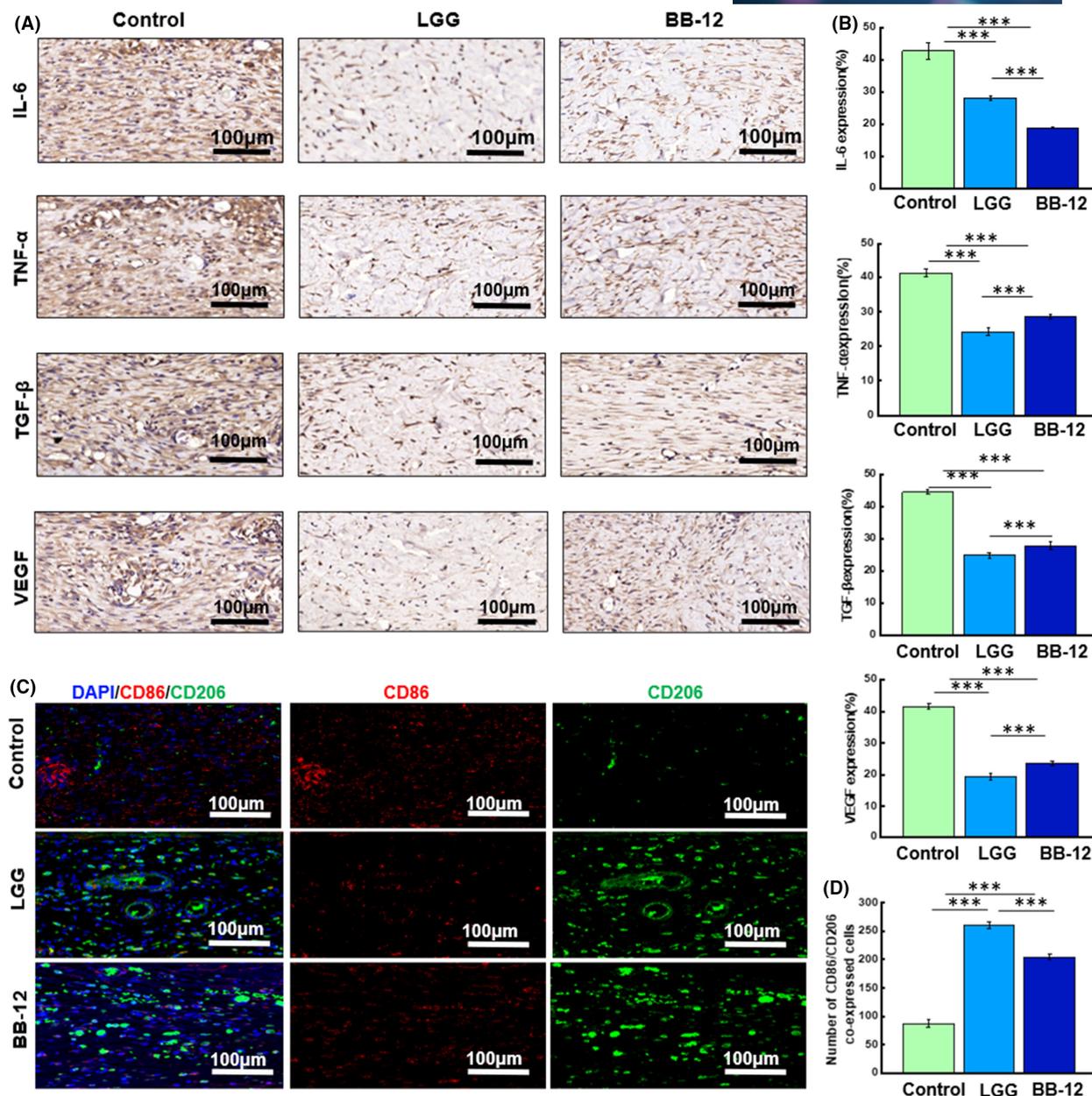
The influence of LGG and BB-12 interventions on the composition of wound microbiota was investigated. There were no significant changes in the richness and diversity of commensal bacteria among the groups, as evidenced by the Chao indices (Figure 5A). Furthermore, the altered microbial structure was examined using principal coordinate analysis (PCoA) plots at the OUT level, which clearly showed the distance (beta diversity) between the three groups. In addition, the PCoA diagram was divided into three cohesive groups, corresponding to the control, LGG and BB-12 groups (Figure 5B).

At the genus level, the Wilcoxon rank-sum test was used to compare the relative abundance of the groups. As illustrated in Figure 5C, the LGG and BB-12 groups showed significant reductions in the abundance of *Staphylococcus* and *Proteus* compared to that in the control group ( $p < 0.05$ ), along with a notable increase in *Corynebacterium* abundance ( $p < 0.05$ ) (Figure 5D). In particular, *Staphylococcus* displayed the most substantial alteration in abundance, accounting for 70.48%, 58.11% and 40.78% in the control, LGG and BB-12 groups respectively. These findings suggest that the application of LGG and BB-12 can effectively modulate the microbial environment in wounds.

## DISCUSSION

This study reveals the significant role of topical probiotics LGG and BB-12 in regulating the infected wound microenvironment for promotion of wound healing. Particular emphasis has been paid to the ability of LGG and BB-12 to reshape the wound microbiota, including inhibiting pathogenic bacteria and promoting the proliferation of beneficial microorganisms, thereby enhancing the natural defence mechanism of the wound, which provides new perspectives and solutions in infected wound treatment. Unlike the proprietary probiotics used in previous studies (Chan et al., 2022; Connell et al., 2018), this study selected LGG and BB-12 which are commonly used, safe and readily available strains, and validates their use in actual wound treatment to promote the widespread use of these common strains in clinical scenario.

Consistent with the findings by Moysidis et al. (2022) and Tarapatzi et al. (2022), our study shows that both LGG and BB-12 significantly enhance the healing of *S. aureus*-infected wounds. Unlike Panagiotou et al.'s (2023) research, we observed that BB-12 outperforms LGG in accelerating wound healing. This advantage may be due to the higher concentrations of short-chain fatty acids (SCFAs), such as acetic acid, propionic acid and butyric acid metabolized by BB-12, which can inhibit *S. aureus* growth and maintain a proper pH for protease activity. In a study by Twetman et al. (2018), the impact of *Lactobacillus reuteri* on matrix metalloproteinases and interferons in oral mucosa wounds showed no significant differences with the placebo interventions. In contrast, this study revealed that the LGG can promote skin wound healing by regulating the pro-inflammatory cytokines, TGF- $\beta$ , VEGF, macrophage polarization and microbiota. The different results with previous studies suggest that



**FIGURE 4** (A) Immunohistochemical staining images and (B) quantification of IL-6, TNF- $\alpha$ , TGF- $\beta$  and VEGF in skin tissue of infected wounds in each group. (C) Immunofluorescence staining images of M1 macrophage marker CD86 (red) and M2 macrophage marker CD206 (green) in wound skin tissue on day 12 in each group. (D) Quantification of CD86 and CD206 in each group.

the effects of probiotics should consider the physiological differences of the tissues they are applied to, as well as the distinct bioactivities of the probiotic strains used.

In the early stages of wound healing, granulation tissue lays the foundation for the formation of new blood vessels and cellular matrix. Previous studies have shown that Bifidobacteria can enhance tissue regeneration by remodelling the extracellular matrix and fibroblast migration (O'Connell Motherway et al., 2019; Silva et al., 2018), as well as promote the differentiation and regeneration of epidermal keratinocytes (Szöllösi et al., 2017). This is consistent with our findings that BB-12 can promote collagen deposition in skin wounds,

which may be due to BB-12 activating key growth factors and signalling pathways, providing a faster and more stable environment for tissue repair. Additionally, several studies (Lam et al., 2007; Mohammedsaeed et al., 2015; Moreira et al., 2021) have shown that *L. rhamnosus* can effectively promote wound healing. The same results have been demonstrated in this study with further evidence that both LGG and BB-12 can promote collagen deposition.

It is known that probiotic strains have specific effects on immune regulation and tissue healing (Yin et al., 2024). Some studies have shown that lactobacilli can reduce the expression of inflammatory factors (Chen et al., 2024; Zanetta et al., 2023) while other



bacterial adhesion and colonization and mitigating harmful metabolites (Chen et al., 2024), thereby improving the wound microenvironment and promoting healing. Notably, BB-12 demonstrated a superior ability to reduce abundance of *Staphylococcus* which is a common pathogen in chronic wound inflammation (Alabbosh et al., 2023; Liu et al., 2022). Furthermore, there is significant reduction in abundance of *Proteus* spp. which is associated with various infections (Kwiecińska-Piróg et al., 2020). The marked increase in *Corynebacterium* abundance suggests a potential protective role in the wound (Oh et al., 2016; Sanford & Gallo, 2013). These results imply that both LGG and BB-12 can not only suppress harmful bacteria but also foster beneficial microbiota to improve the wound microenvironment. The superior performance of BB-12 in controlling pathogenic bacteria and LGG in promoting beneficial bacteria highlights the specific roles in wound microbiota regulation. This underscores the importance of selecting appropriate probiotics based on microbiota characteristics for wound management. Future studies should further investigate the comprehensive effects of these probiotics on wound healing to optimize clinical application strategies.

## CONCLUSIONS

This study highlights the significant role of topical probiotics of LGG and BB-12 in modulating the wound microenvironment to promote wound healing. It is evidenced that both LGG and BB-12 improved wound healing with enhanced granulation tissue formation and collagen deposition. The neutrophil infiltration and the expression of pro-inflammatory cytokines have been inhibited by both probiotics. Notably, BB-12 markedly reduced IL-6, while LGG significantly lowered TNF- $\alpha$ , TGF- $\beta$  and VEGF. Additionally, the probiotics intervention promoted macrophage polarization towards the anti-inflammatory M2 phenotype. Notably, LGG and BB-12 significantly decreased the abundance of pathogenic bacteria (e.g. *Staphylococcus* and *Proteus*) and increased the proportion of beneficial bacteria (e.g. *Corynebacterium*). Particularly, BB-12 was more effective in reducing *Staphylococcus* abundance, whereas LGG excelled in promoting *Corynebacterium* growth. These results provide new theoretical support for probiotic-based wound treatment strategies for their broader application in clinical practice. Despite the important outcomes, certain limitations exist, such as the reliance on animal models and the lack of coverage for all types of wounds and clinical conditions. Future research should focus on expanding sample sizes, covering various wound models and clinical applications and exploring the specific roles and mechanisms of different probiotic strains in modulating the microbiota. This will help further optimize probiotic therapies, enhance

wound care outcomes and offer new solutions for treating complex infections and injuries.

## AUTHOR CONTRIBUTIONS

**Zhe Yin:** Data curation; formal analysis; investigation; methodology; writing – original draft. **Yilin Wang:** Data curation; formal analysis; investigation. **Xiaojuan Feng:** Investigation; methodology. **Changqing Liu:** Methodology. **Shuyan Liu:** Formal analysis; investigation. **Xiaoyang Guan:** Formal analysis. **Zhanyi Long:** Data curation; investigation. **Zhonghua Miao:** Software. **Fang He:** Formal analysis. **Ruyue Cheng:** Methodology. **Yanting Han:** Funding acquisition; investigation; methodology; writing – original draft; writing – review and editing. **Ka Li:** Funding acquisition; project administration; supervision.

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## CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

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## SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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