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Microbiota-Derived Metabolites Regulate Bacterial Virulence and Tissue Repair Pathways in a Non-Invasive Surgical Model

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Abstract

Background: Microbiota-derived metabolites play an important role in regulating bacterial behavior and host tissue repair. Understanding their interaction may provide new approaches to improve healing in veterinary surgery. **Aim:** This study evaluated the relationship between short-chain fatty acids (SCFAs), bacterial biofilm gene expression, and host collagen synthesis using a controlled experimental model. **Methods:** Fifteen mice were randomly divided into three groups (n = 5 per group): control, SCFA-rich diet, and dysbiosis. Skin response was evaluated using a non-invasive model. The bacterial gene icaA and host gene COL1A1 were analyzed using RT-qPCR. SCFAs levels were measured using standard biochemical assays. Data were expressed as mean ± SE and analyzed using one-way ANOVA. **Results:** The SCFA-rich group showed a significant reduction in icaA expression (0.42 ± 0.03) compared with control (1.00 ± 0.05) and dysbiosis (1.68 ± 0.07) groups ($p < 0.05$; $p < 0.001$). In contrast, COL1A1 expression was significantly increased in the SCFA-rich group (2.35 ± 0.10) compared with control (1.00 ± 0.06) and dysbiosis (0.62 ± 0.04) groups ($p < 0.05$; $p < 0.001$). SCFAs levels were higher in the SCFA-rich group (82 ± 3) and lower in the dysbiosis group (28 ± 2) compared with control (45 ± 2) ($p < 0.05$; $p < 0.001$). **Conclusion:** SCFAs were associated with reduced bacterial biofilm gene expression and enhanced collagen synthesis, suggesting a key role in improving tissue repair. These findings support the potential use of microbiota-derived metabolites as a non-invasive strategy to enhance healing outcomes in veterinary surgery.

Keywords: Biofilm, COL1A1, dysbiosis, icaA, microbiota, SCFAs, wound healing.

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Introduction

The complexities of wound healing involve biological factors that include both responsive host tissue and microbial interactions. Recent studies have focused attention on the importance of certain microbiota and their metabolites and how they may assist these processes. Many of these studies have proven the

antimicrobial defense and tissue repair mechanisms synergistically activated by microbial-derived compounds (Dinić et al., 2024; Lima et al., 2025).

Furthermore, studies have shown that postbiotics metabolites can enhance the protective function of the skin barrier and decrease the bacterial invasion, particularly when the bacteria are Staphylococcus aureus, a common bacterium that infects wounds (Dinić et al., 2024). In addition, wound healing has shown to be positively affected by the tissue regenerative and infective controlling properties of certain beneficial bacterial metabolites that have been integrated into dressings (Han et al., 2024).

Lysates and postbiotics have been shown to affect immune responses and increase the activity of keratinocytes and this has been shown to affect wound healing positively (Altves et al., 2024; Heo et al., 2024). It has been proven that the metabolites of certain microbiota are a key component in the processes of chronic and pathological wounds as well as in the diabetic wounds and the aberrant scar tissue formation (Huang et al., 2025; Jin & Zheng, 2025).

The interactions of microbiota metabolites, expression of virulence genes of bacteria, and synthesis of collagen by the host remain therefore poorly understood. This study aims to assess the impact of metabolites from microbiota on the regulation of biofilm formation by bacteria and the repair of host tissues, using a simplified experimental model.

Materials and Methods

Experimental Animals and Study Design

Fifteen healthy male mice aged 6-8 weeks, weighing 25-30 g, were obtained from the animal house facility of the College of Veterinary Medicine. The animals were kept in a controlled environment of 22 ± 2°C, 55 ± 5% relative humidity and a 12:12 light/dark cycle and had ad libitum access to standard feed and water. The mice were randomly assigned to three different groups (n = 5 for each group). The control group was fed a standard diet, the SCFA-rich group was given a diet designed to increase short-chain fatty acid production, and the dysbiosis group was given broad-spectrum antibiotics to perturb their microbiota. The experimental period lasted for 21 days. All procedures performed followed institutional ethical standards (Approval No. 626).

Induction of Non-Invasive Skin Model

A non-invasive skin model was developed using a tape-stripping method that simulates superficial skin stress without requiring a surgical approach. For this, an adhesive tape was applied to a designated dorsal skin site (2 \times 2 cm) and removed 10 times. This technique caused a controlled loss of stratum corneum that did not elicit bleeding, thereby permitting the study of the dermal molecular response to injury and subsequent healing processes.

Sample Collection

At the endpoint of the study, appropriate anesthesia was administered before the humane euthanization of the subjects, and skin samples from the experimental area were collected using sterile gloves. Each skin sample was divided into sections designated for molecular and biochemical analyses. The sections were sealed and placed in liquid nitrogen to snap freeze, and then stored at −80°C to preserve the integrity of RNA and the stability of metabolites.

RNA Extraction and cDNA Synthesis

TRIzol™ Reagent (Invitrogen, Thermo Fisher Scientific, USA) was used to obtain total RNA from skin tissue samples. Manufacturer's protocol was strictly followed. Purity and total RNA concentration was measured using a NanoDrop™ 2000 (Thermo Fisher Scientific, USA). After, cDNA was synthesized using one (1) microgram (1 µg) of total RNA and RevertAid First Strand cDNA Synthesis Kit (Thermo Scientific, Lithuania). Total reaction volume was 20 µL. Instructions provided in the kit were followed.

Quantitative Real-Time PCR (RT-qPCR)

SYBR™ Green PCR Master Mix (Applied Biosystems, USA) was used to analyze gene expression along with the Applied Biosystems QuantStudio™ 5 Real-Time PCR System (Applied Biosystems, USA) to analyze the expression of the selected genes icaA, a gene of interest, and host gene COL1A1, plus the internal control gene, expressed in all bacteria, 16S rRNA, and the host gene control gene, GAPDH. Amplification protocol was set to 95°C for 5 minutes for gel denaturation and then 40 cycles of 95°C for 15 second (s) to denature the gel, 55–60°C for 30 s to anneal the primers and 72°C for 30 seconds (s) to extend the strand. Relative gene expression levels were computed utilizing the $2^{-}\Delta\Delta Ct$ method (Table 1).

Table 1: Primers used in the study

Gene	Function	Forward Primer (5′→3′)	Reverse Primer (5′→3′)	Amplicon (bp)	Reference
icaA	Biofilm formation	GAGGTAAAGCCAACGCACTC	CCTGTAACCGCACCAAGTTT	151	Atshan et al., 2013
COL1A1	Collagen synthesis	GGTATGCTTGATCTGTATCTG	AGTCCAGTTCTTCATTGCATT	138	Chen et al., 2017
GAPDH	Host reference gene	TCACCACCATGGAGAAGGC	GCTAAGCAGTTGGTGGTGCA	150	Chen et al., 2017

Measurement of Short-Chain Fatty Acids (SCFAs)

SCFA concentration from tissue samples was evaluated using a commercial ELISA kit (MyBioSource Inc., USA) following kit instructions. Absorbance readings at 450 nm were taken using a microplate reader (BioTek Instruments, USA) and results were calculated as \u00b5mol per gram of tissue.

Statistical Analysis

Data were presented as mean \u00b1 standard error (SE). For all statistical analysis, SPSS software version 26.0 (IBM Corp., USA) was utilized. For group comparisons, a one-way analysis of variance (ANOVA) followed by post hoc testing was performed. Statistically significant results were noted at $p < 0.05$ and at $p < 0.001$, results were considered highly significant.

Results

Bacterial Gene Expression (icaA)

There is a highly notable difference in expression of the gene icaA that relates to bacterial biofilms between the experimental groups. The SCFA-rich group has a 0.42 ± 0.03 value of expression which is much lower than the 1.00 ± 0.05 value of the control group. On the other side, the expression of icaA in the dysbiosis group is notable as its 1.68 ± 0.07, higher than all other groups. After statistical analysis, it is determined that there are significant differences between the control group, SCFA-rich group, and dysbiosis group, with ($p < 0.05$) and the SCFA-rich group has a highly significant ($p < 0.001$) as the expression is significantly lower than both the control and dysbiosis groups, and the dysbiosis group is significantly higher than the control group (Figure 1).

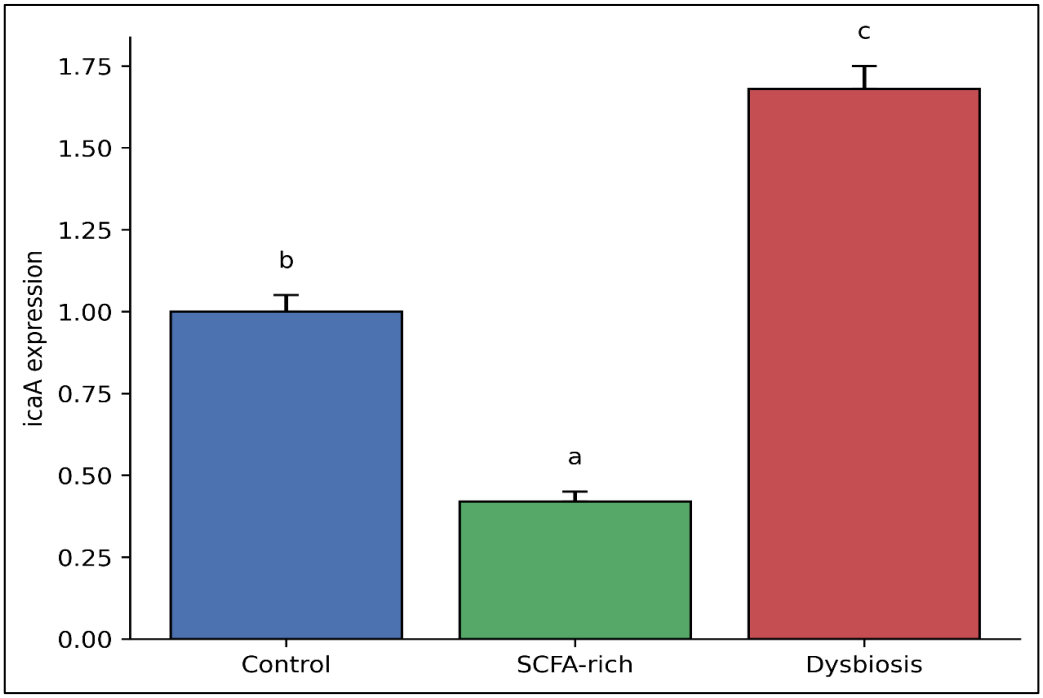

Figure 1: Relative expression of bacterial icaA gene among experimental groups

Host Gene Expression (COL1A1)

Among the groups there is a very notable difference in the expression of the gene COL1A1 that relates to the host collagen. The expression of COL1A1 gene is significantly increased in the SCFA-rich experimental group with 2.35 ± 0.10 value, much higher than that of the control group at 1.00 ± 0.06. In contrast, the expression of the same gene in the dysbiosis group is 0.62 ± 0.04, which is significantly lower than all other groups. Among the groups there is a significant difference in expression of the groups ($p < 0.05$) and the level is highly significant with ($p < 0.001$) as the SCFA-rich group has the highest expression, and the expression in the dysbiosis group is the lowest (Figure 2).

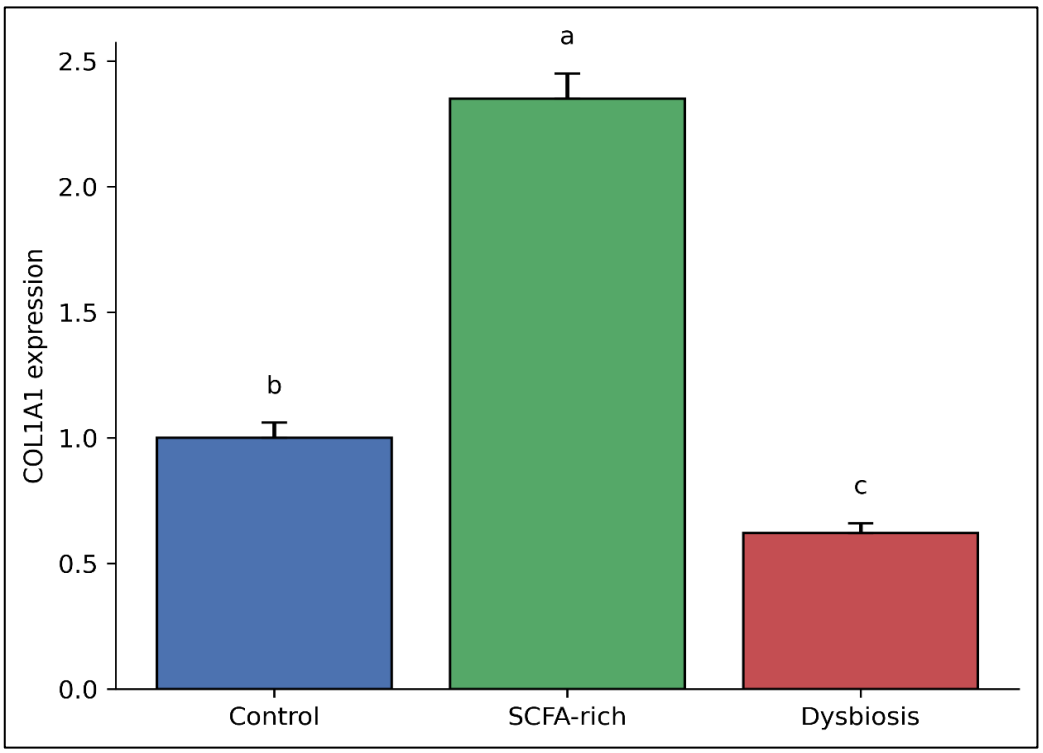

Figure 2: Relative expression of host COL1A1 gene among experimental groups

Short-Chain Fatty Acids (SCFAs)

A distinct pattern was observed in short-chain fatty acid levels in all groups. The SCFA-rich group exhibited the greatest levels (82 ± 3) compared to the control group (45 ± 2) and the dysbiosis group, which exhibited the lowest levels (28 ± 2). Given the strong, significant differences ($p < 0.001$) among control, SCFA-rich, and dysbiosis groups, SCFAs were linked to enhanced tissue repair molecular markers in the SCFA-rich group and absent tissue repair responses in dysbiosis (Figure 3).

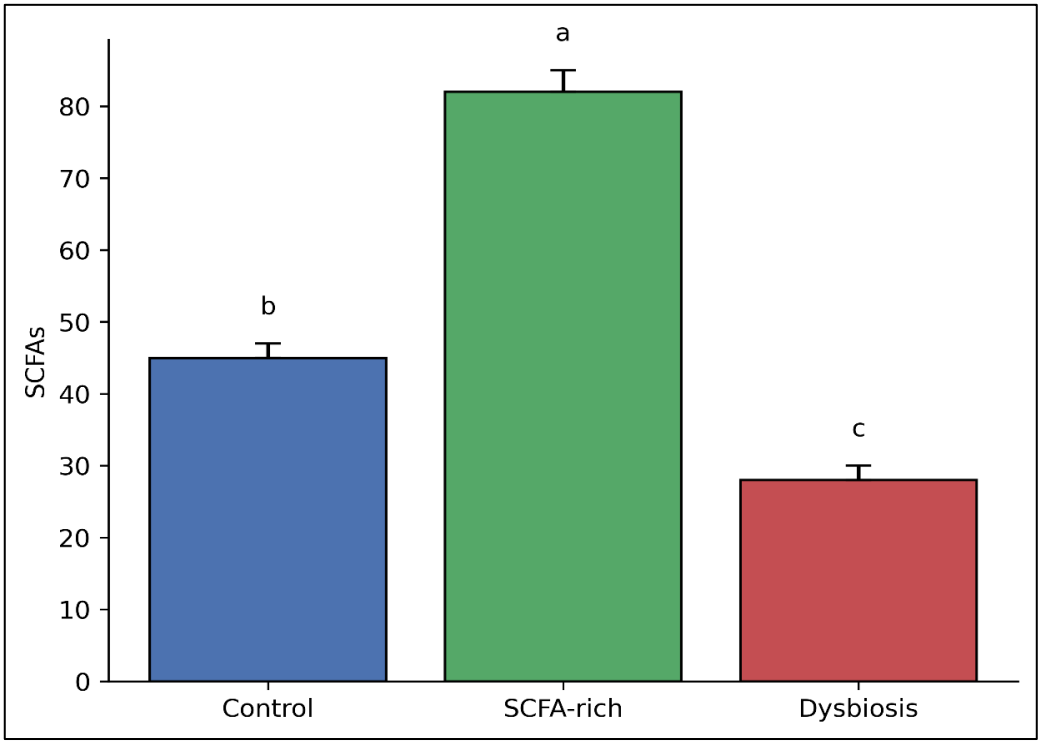

Figure 3: Levels of short-chain fatty acids (SCFAs) in experimental groups

Discussion

The findings of the present study show that SCFAs along with other metabolites of the microbiome were associated with lowered bacterial biofilm gene expression and augmented collagen production by the host. The reduction of icaA expression in the SCFA-rich group indicates lower biofilm production which is one of the major contributors to persistent infections in wounds. This finding is in line with earlier reports stating that postbiotics along with microbial metabolites provide pathogenic bacteria with an enhanced defense against the bacteria and an improved antimicrobial defense (Hashemi et al., 2025; Prajapati et al., 2025).

The SCFA-rich group also had significantly higher collagen production and tissue repair, shown by the increased expression of COL1A1. This is supported by data that shows polymerization of extracellular matrix is increased by compounds of microbiota (Zhang et al., 2025). In addition, the decrease in SCFA of the dysbiosis group and the increase in bacterial gene expression, provide evidence that an imbalance of the microbiota compromises both the microbial control and the regeneration of tissue. Disruption of the metabolites derived from microbiota similarly impaired the functioning of skin barrier and its healing potential (Wang et al., 2025; Zeng et al., 2025).

The dysbiosis group has heightened levels of icaA expression, which could indicate more pronounced virulence and interspecies interactions among the bacteria in the wound microenvironment. Bacterial crosstalk and the expression of virulence factors have previously been demonstrated to significantly contribute to the impairment of wound healing (Daher et al., 2025; Hammad et al, 2025).

Conclusion

The findings demonstrate the importance of microbiome metabolites in the modulation of bacterial activities and the stimulation of the host's healing processes. This effect outlines a possible interventional approach to enhance healing outcomes.

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