J Korean Med Sci. 2025 Dec 15;40(48):e327
https://doi.org/10.3346/jkms.2025.40.e327
eISSN 1598-6357·pISSN 1011-8934

JKMS

Original Article

Clinical Medicine in Other Specialties

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Efficacy and Safety of Human Skin Microbiome-Derived Strains as Topical Treatment for Acne: An In Vitro and In Vivo Study

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OPEN ACCESS

Received: Apr 2, 2025
Accepted: Jun 24, 2025
Published online: Nov 20, 2025

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ABSTRACT

Background: The skin microbiome plays a crucial role in defending against pathogens and modulating immunity, and its dysregulation is linked to various skin conditions, including acne.
Methods: In this study, four previously identified strains—*Staphylococcus epidermidis* B424F-5, *S. epidermidis* BS47C-1, *Dermacoccus profundi* BS35F-3, and *Streptococcus salivarius* BS320F-4— were selected from a skin microbiome database of healthy individuals. The efficacy and safety of these strains against acne-related inflammation were evaluated using in vitro and in vivo animal model experiments.
Results: *Cutibacterium acnes* exposure increased the expression of acne-associated inflammatory mediators—such as IL-1β, IL-6, IL-8, COX-2, iNOS, and TNF-α—particularly in keratinocytes, without inducing cytotoxicity. Treatment with heat-killed *S. epidermidis* BS47C-1 (SE2), *D. profundi* BS35F-3 (DP), and *S. salivarius* BS320F-4 (SS) significantly reduced these markers in vitro. In vivo, topical application of the strains alleviated inflammation in a *C. acnes*-induced mouse model, with histological evidence of reduced erythema and immune cell infiltration. Bulk RNA sequencing of keratinocytes showed that SE2 and DP downregulated cytokine and interferon signaling while enhancing skin barrier and antimicrobial gene expression, suggesting a dual anti-inflammatory and barrier-supporting mechanism.
Conclusion: These results provide compelling evidence of the efficacy and safety of human skin microbiome-derived strains as potential topical treatments for acne. By targeting both microbial colonization and inflammatory pathways, these strains offer a promising avenue for the development of novel acne therapeutics.

Keywords: Acne Vulgaris; *Cutibacterium acnes*; Inflammation; Microbiome

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Funding

This work was supported by the Technology Innovation Program (or Industrial Strategic Technology Development Program-Novel microbiome therapy for hidradenitis suppurativa and severe acne based on integrative microbiome-multiomics approach) (20024192) funded By the Ministry of Trade, Industry & Energy (MOTIE, Korea).

Disclosure

The authors have no potential conflicts of interest to disclose.

Author Contributions

Conceptualization: Chu J, Lee YI. Formal analysis: Lee SG, Lee JH. Investigation: Lee SG. Methodology: Chu J, Kim KH, Lee YI. Project administration: Kim KH, Shin CH, Lee YI. Resources: Chu J, Kim KH, Shin CH. Software: Lee SG, Lee JH. Supervision: Lee YI. Validation: Ham S. Visualization: Lee SG, Lee JH, Baek Y. Writing - original draft: Lee SG, Lee JH, Lee YI. Writing - review & editing: Lee SG, Lee YI.

INTRODUCTION

Acne vulgaris is a chronic inflammatory disorder of the pilosebaceous unit that affects up to 90% of adolescents and young adults.[1] The etiopathogenesis of acne is multifactorial, involving increased sebum production, follicular hyperkeratinization, and the complex interplay between inflammatory mediators and microbial factors.[2] Bacterial colonization exacerbates the pathology as a secondary factor, aggravating disease severity.[3] Dysbiosis of the microbiome (an imbalance in beneficial commensal microorganisms and pathogens) is associated with a variety of skin diseases.[4] Notably, in acne, specific subgroups, namely *Cutibacterium acnes* phylotypes IA-2, IB-1, and IC, are detected more frequently in individuals with severe acne than in those with normal skin.[3]

Recent advances in microbiome research have underscored the importance of the skin microbial ecosystem in maintaining cutaneous homeostasis.[5] Emerging evidence indicates that changes in the relative abundance and virulence of skin microbes (including not only *C. acnes* but also organisms such as *Enterococcus faecalis*) can significantly influence acne pathogenesis.[4] Skin microorganisms act in a manner similar to their counterparts in the gut; they exhibit protective functions against pathogenic infections, participate in immune modulation, and contribute to the breakdown of various biomolecules, including lipids, proteins, and complex carbohydrates. Conventional treatments, such as topical and systemic antibiotics, primarily target bacterial colonization and inflammation but are associated with treatment risks such as antibiotic resistance and adverse effects. In contrast, microbiome-based therapies have shown promise in clinical studies by achieving comparable efficacy to conventional methods while potentially offering improved safety profiles.[6]

In a previous study, we successfully collected skin swab samples from 51 healthy individuals and constructed a comprehensive database of skin microbiome data.[7] Over 1,630 strains of skin-derived microorganisms were isolated. Furthermore, antimicrobial activities against skin and opportunistic pathogens, such as *Staphylococcal aureus, Bacillus subtilis,* and *C. acnes* were assessed based on these strains and their derived antimicrobial substances (bacteriocins). These experiments identified four skin microbiome-derived strains *Staphylococcus epidermidis* B424F-5 (SE1), *Staphylococcus epidermidis* BS47C-1 (SE2), *Dermacoccus profoundi* BS35F-3 (DP), *Streptococcus salivarius* BS320F-4 (SS) exhibiting antimicrobial activity against *C. acnes*.

Building on these findings, the present study aims to evaluate the therapeutic potential and safety of heat-killed candidate strains (SE1, SE2, DP, and SS) and their antimicrobial metabolites through comprehensive preclinical in vitro and in vivo studies. We hypothesize that these strains exert their therapeutic effects by modulating acne-related skin inflammation. Using both cellular models and an acne-induced animal model, this study seeks to demonstrate their anti-inflammatory efficacy in the context of acne pathogenesis and to lay the groundwork for the development of a novel, safe, microbiome-based therapeutic approach for acne management.

METHODS

Bacterial culture

C. acnes ATCC 6919 (ATCC, Manassas, VA, USA) was cultured in Reinforced Clostridial Medium (Oxoid, Hampshire, UK) under anaerobic conditions at 37°C for 72 hours. Four

candidate strains —SE1, SE2, DP, and SS — were cultured aerobically at 37°C for 24 hours using Tryptic Soy Broth (SE1, SE2), Brain Heart Infusion Medium (SS), or Trypticase Soy Yeast Extract Medium (DP). The antimicrobial activity of these strains was previously characterized, with details provided in our prior publication.[5] Following culture, bacterial suspensions were centrifuged (14,000 rpm, 20 minutes, 4°C), washed twice with PBS, and adjusted to an optical density of 1.0 at 600 nm. Final concentrations were standardized to 1×10^7 CFU in 50 μL. Strains used for experiments were freshly prepared weekly and stored at 4°C, with daily administration during the study period.

Cell culture

Human dermal fibroblasts (HDF; C0135C, Thermo Fisher Scientific, Waltham, MA, USA) and human epidermal keratinocytes (KC; PCS-200-011, ATCC) were cultured in Dulbecco's Modified Eagle Medium (DMEM; Lonza, Walkersville, MD, USA) with 10% fetal bovine serum (Gibco, Waltham, MA, USA) and 1% penicillin-streptomycin (Gibco) or KBM Gold Basal Medium (Lonza) supplemented with KGM Gold SingleQuot (Lonza), respectively. Cells were maintained at 37°C in a humidified incubator with 5% CO_2 and subcultured every three days using Trypsin-EDTA.

Cell viability assay

Cell viability was assessed using the CCK-8 assay (Dojindo Molecular Technologies, Kumamoto, Japan). KC and HDF cells (5×10^4 cells/well) were seeded in 96-well plates and incubated for 24 hours. *C. acnes* were added at varying concentrations (0.25, 0.5, 1, 2, or 4×10^8 CFU), and absorbance was measured at 450 nm using a microplate reader (VersaMax; Molecular Devices, San Jose, CA, USA).

In vivo *C. acnes*-induced acne model and treatment

Six-week-old female CD-1 mice (OrientBio, Seongnam, Korea) were acclimated for one week and randomized into five groups (n = 5 per group). Mice were maintained under standard conditions (24°C ± 0.5°C; 55–65% humidity; 12-hour light/dark cycle) with ad libitum access to food and water. The acne model was established by daily intradermal injections of *C. acnes* (1×10^7 CFU in 20 μL) on both sides of the shaved dorsal skin for two weeks.[8,9] Subsequently, heat-killed bacterial suspensions (50 μL per strain) were topically applied once daily for 7 consecutive days. The bacterial preparations were suspended in the same culture medium used during strain cultivation, without any additional vehicle or carrier. To enhance absorption and ensure even distribution, the treated area was gently tapped at least 40 times using a sterile plastic spatula immediately after application, while mice were under brief inhalational anesthesia. Skin inflammation was documented at baseline, week 2, and week 3. Mice were sacrificed on day 22 via CO_2 inhalation.

Histopathology

Skin samples were collected, fixed in 10% neutral-buffered formalin, embedded in paraffin, and sectioned at 4 μm thickness. Hematoxylin-stained sections were analyzed for epidermal and dermal thickness, measured as described previously.[10,11]

Immunohistochemistry

Paraffin-embedded sections (4 μm) underwent heat-induced epitope retrieval in EnV FLEX TRS High pH buffer (S2367, Dako, Glostrup, Denmark) for 30 minutes. Endogenous peroxidase was quenched with 3% hydrogen peroxide on ice. After blocking with 5% bovine serum albumin, sections were incubated overnight at 4°C with primary

https://doi.org/10.3346/jkms.2025.40.e327

antibodies against nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) (1:200), IL-1β (1:500), and Caspase-1 (1:200) (Cell Signaling). Detection was performed using a DAKO peroxidase/DAB kit, followed by counterstaining with Mayer's hematoxylin. Detailed information on the immunohistochemical studies and materials are presented in the supplementary materials and methods (**Supplementary Data 1**).

Quantitative reverse transcription-polymerase chain reaction (qRT-PCR)

To assess anti-inflammatory effects, keratinocytes were stimulated with *C. acnes* (1×10^7 CFU), followed by co-treatment with heat-killed SE1, SE2, SS, or DP strains (1×10^7 CFU each) for 24 hours. Total RNA from keratinocytes and tissue samples was extracted using RNAiso Plus (Takara Bio, Shiga, Japan), with homogenization of tissue biopsies in 0.9% saline (1 mL per 3 × 3 mm). cDNA was synthesized using the RNA to cDNA EcoDry™ premix kit (Takara Bio). Gene expression was quantified by qRT-PCR using SYBR Green Master Mix (Promega) on a QuantStudio 3 Real-Time PCR System (Applied Biosystems, Foster City, CA, USA). Expression levels were normalized to GAPDH and calculated using the $2^{-\Delta\Delta Ct}$ method. Primer sequences are provided in **Table 1**.

RNA sequencing (RNA-seq)

RNA sequencing was performed following total RNA extraction with RNAiso Plus (Takara Bio) and library preparation using a TruSeq RNA Library Prep Kit (Illumina). Paired-end sequencing (100 bp) was conducted on the NextSeq 550 platform (Macrogen, Seoul, Korea).

Table 1. Sequence of primers for qRT-PCR for mouse and human genes

Variables	Primer sequences (5′→3′)
Mouse	
Tnfα	Forward: 5′-GGTGCCTATGTCTCAGCCTCTT-3′
	Reverse: 5′-GCCATAGAACTGATGAGAGGGAG-3′
Il1β	Forward: 5′-TGGACCTTCCAGGATGAGGACA-3′
	Reverse: 5′-GTTCATCTCGGAGCCTGTAGTG-3′
Il6	Forward: 5′-TACCACTTCACAAGTCGGAGGC-3′
	Reverse: 5′-CTGCAAGTGCATCATCGTTGTTC-3′
Il8	Forward: 5′-GGTGATATTCGAGACCATTTACTG-3′
	Reverse: 5′-GCCAACAGTAGCCTTCACCCAT-3′
Cox2	Forward: 5′-GCGACATACTCAAGCAGGAGCA-3′
	Reverse: 5′-AGTGGTAACCGCTCAGGTGTTG-3′
inos	Forward: 5′-GAGACAGGGAAGTCTGAAGCAC-3′
	Reverse: 5′-CCAGCAGTAGTTGCTCCTCTTC-3′
Gapdh	Forward: 5′-AGGTCGGTGTGAACGGATTTG-3′
	Reverse: 5′-TGTAGACCATGTAGTTGAGGTCA-3′
Human	
TNFα	Forward: 5′-CTCTTCTGCCTGCTGCACTTTG-3′
	Reverse: 5′-ATGGGCTACAGGCTTGTCACTC-3′
IL1β	Forward: 5′-CCACAGACCTTCCAGGAGAATG-3′
	Reverse: 5′-GTGCAGTTCAGTGATCGTACAGG-3′
IL6	Forward: 5′-AGACAGCCACTCACCTCTTCAG-3′
	Reverse: 5′-TTCTGCCAGTGCCTCTTTGCTG-3′
IL8	Forward: 5′-GACCACACTGCGCCAACAC-3′
	Reverse: 5′-CTTCTCCACAACCCTCTGCAC-3′
COX2	Forward: 5′-CGGTGAAACTCTGGCTAGACAG-3′
	Reverse: 5′-GCAAACCGTAGATGCTCAGGGA-3′
iNOS	Forward: 5′-GCTCTACACCTCCAATGTGACC-3′
	Reverse: 5′-CTGCCGAGATTTGAGCCTCATG-3′
GAPDH	Forward: 5′-TGTTGCCATCAATGACCCCTT-3′
	Reverse: 5′-CTCCACGACGTACTCAGCG-3′

qRT-PCR = quantitative reverse transcription-polymerase chain reaction, TNFα = tumor necrosis factor-alpha, IL = interleukin, COX2 = cyclooxygenase2, iNOS = inducible nitric oxide synthase.

https://doi.org/10.3346/jkms.2025.40.e327

Reads were trimmed and mapped to the reference genome using HISAT2. Transcript assembly and quantification were performed using StringTie, generating read counts, FPKM, and TPM values. Differentially expressed genes were identified using DESeq2 with thresholds of |fold change| ≥ 2 and $P < 0.05$.

Statistical analysis

Data are presented as mean ± standard deviation. Statistical analyses were performed using SPSS (version 25.0; IBM Corp., Armonk, NY, USA). For two-group comparisons, unpaired *t*-tests were used when normality and homogeneity of variance were confirmed. Comparisons between each treatment and the *C. acnes*-only group were performed using unpaired *t*-tests. For multiple comparisons, one-way analysis of the variance followed by Bonferroni post-hoc tests was conducted. A *P* value < 0.05 was considered statistically significant. Principal component analysis (PCA) and hierarchical clustering analysis (HCA) of transcriptomic data were performed using rlog-transformed values, with results visualized through heatmaps and PCA plots.

Ethics statement

All animal experiments were conducted in accordance with the ARRIVE guidelines and approved by the Institutional Animal Care and Use Committee of Yonsei University (IACUC approval No. 2023-0111).

RESULTS

In vitro anti-inflammatory effects of candidate strains

The cytotoxic effects of *C. acnes* on KCs and HDFs were evaluated using a CCK-8 assay. As shown in **Fig. 1**, both KCs and HDFs demonstrated increased cell viability with rising concentrations of *C. acnes* (10^6 to 10^8 CFU), indicating no cytotoxicity in either cell type. To assess changes in inflammatory mediators associated with acne pathogenesis (interleukin 1 beta [*IL1β*], interleukin 6 [*IL6*], interleukin 8 [*IL8*], tumor necrosis factor alpha [*TNFα*], inducible nitric oxide synthase [*iNOS*], and cyclooxygenase-2 [*COX-2*]), qRT-PCR was performed after exposing KCs and HDFs to *C. acnes* (1×10^7 or 5×10^7 CFU). In KCs, *C. acnes* exposure led to significant upregulation of *IL1β, IL6, IL8,* and *COX-2* expression compared to controls (**Supplementary Fig. 1A**). In HDFs, while there was some increase in inflammatory mediator expression, these changes were inconsistent and not correlated with *C. acnes* dose (**Supplementary Fig. 1B**). These findings suggest that keratinocytes may play a key role in mediating acne-related inflammatory responses under these experimental conditions.

Subsequently, qRT-PCR analysis was conducted to determine whether the four candidate strains could attenuate *C. acnes*-induced inflammatory responses. Treatment with SE2, SS, and DP significantly reduced the expression of *IL1β, IL6, IL8, COX-2, iNOS*, and *TNFα* compared to *C. acnes*-only exposure in KCs (**Fig. 2**). In contrast, SE1 showed relatively inconsistent effects and was therefore excluded from subsequent RNA-seq and in vivo experiments.

Transcriptomic analysis by RNA sequencing

RNA sequencing was performed on keratinocytes exposed to *C. acnes* (Acne group) and treated with each candidate strain (SE2, SS, and DP groups). PCA revealed distinct clustering of samples based on treatment conditions, indicating clear transcriptional differences

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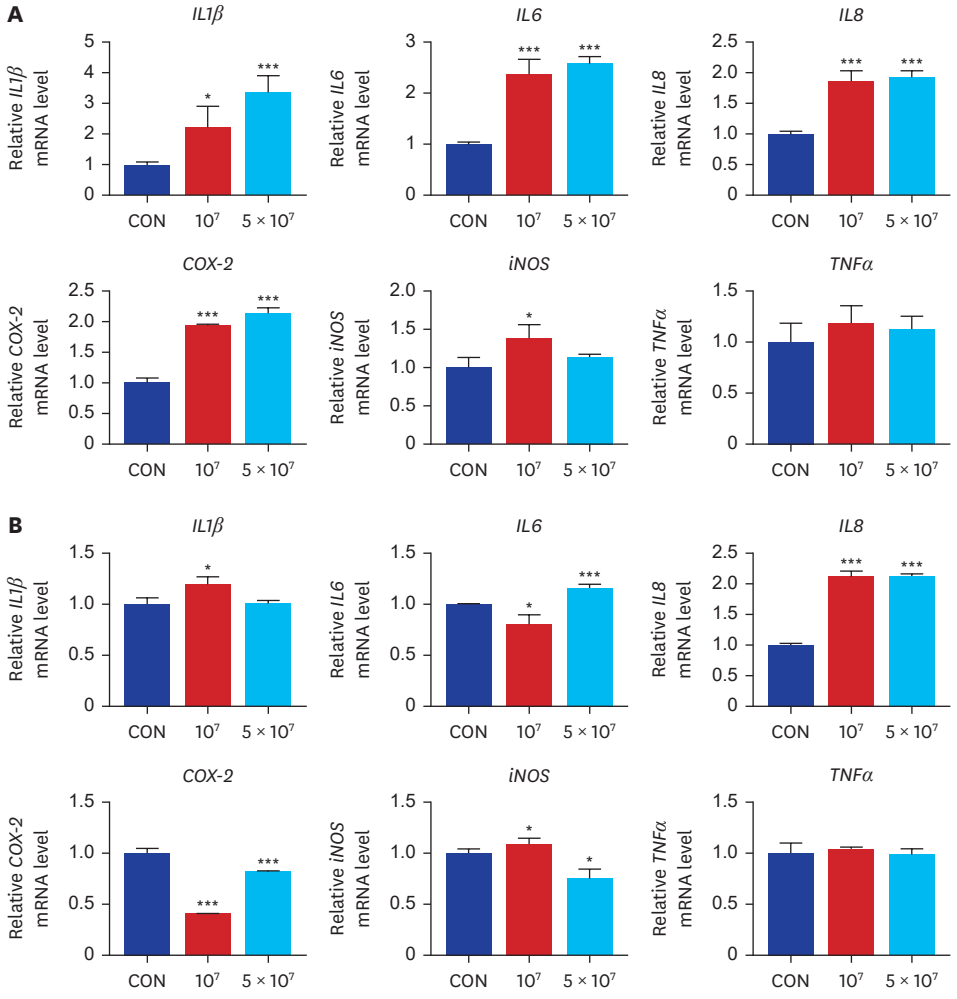

Fig. 1. Reverse transcription-polymerase chain reaction analysis of inflammatory markers in vitro following *Cutibacterium acnes* exposure. *C. acnes* was applied to KCs (**A**) and HDFs (**B**) to assess mRNA expression of acne-associated inflammatory mediators. A dose-dependent increase in *IL1β*, *IL6*, *IL8*, and *COX-2* expression was observed in KCs but not in HDFs. Gene expression was normalized to glyceraldehyde 3-phosphate dehydrogenase and calculated using the $2^{-\Delta\Delta Ct}$ method.
IL = interleukin, CON = control, COX-2 = cyclooxygenase-2, iNOS = inducible nitric oxide synthase, TNFα = tumor necrosis factor-α, KC = keratinocyte, HDF = human dermal fibroblast.
$^{*}P < 0.05$, $^{***}P < 0.001$, independent samples *t*-test vs. control.

(**Fig. 3A**). HCA of the 1,831 most significantly altered genes further visualized these differences in a heatmap (**Fig. 3B**). Among the three strains, gene set enrichment analysis (GSEA) demonstrated that SE2 treatment efficiently upregulated genes involved in skin barrier function and antimicrobial responses, while downregulating genes associated with cytokine signaling and interferon pathways compared to the Acne group (**Fig. 3C**). The DP group exhibited similar transcriptional changes (**Fig. 3D**). These results emphasize condition-specific transcriptional shifts induced by SE2 and DP, underscoring their biological relevance and potential anti-inflammatory effects.

In vivo effects of candidate strains on *C. acnes*-induced inflammation

In vivo evaluation and histopathology

A mouse acne model was established by intradermal injection of *C. acnes* (1×10^7 CFU in 20 μL) for two weeks (**Fig. 4A**). The Acne group exhibited persistent swelling and erythema at

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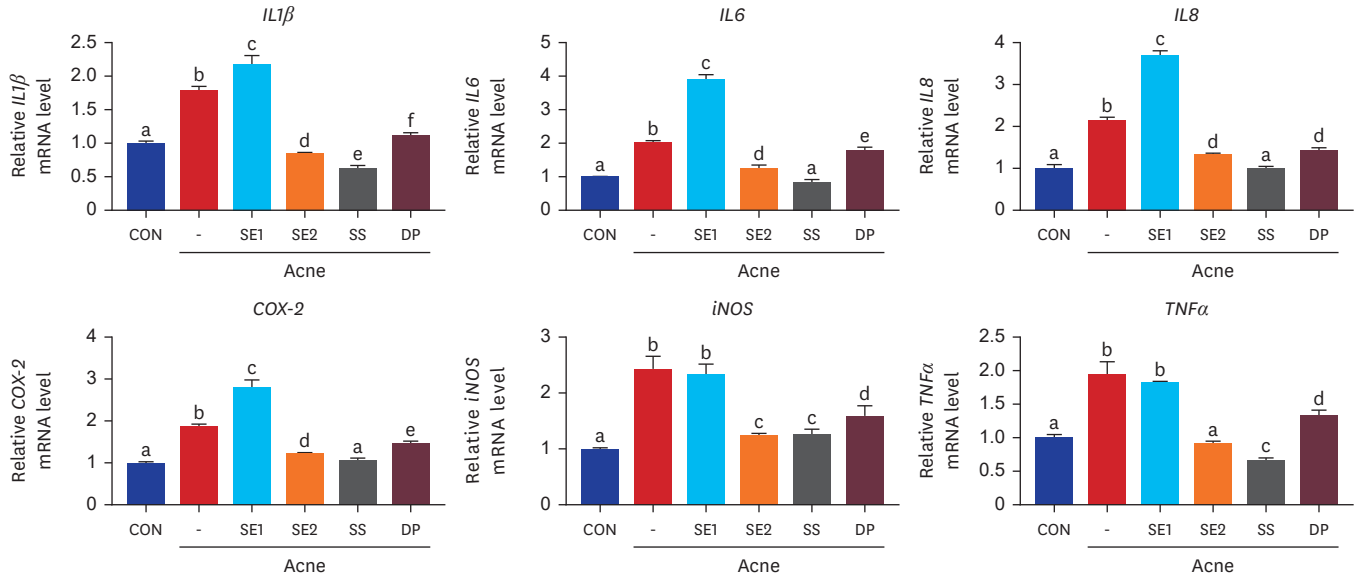

Fig. 2. Reverse transcription-polymerase chain reaction analysis of inflammatory markers in KCs treated with *Cutibacterium acnes* and four candidate strains. KCs were exposed to *C. acnes* alone (Acne group) or co-treated with SE1, SE2, DP, or SS. SE2, SS, and DP significantly reduced the expression of *IL1β*, *IL6*, *IL8*, *TNFα*, *iNOS*, and *COX-2* compared to the Acne group, while SE1 showed no such effect. Data are mean ± SD. Different lowercase letters (a, b, c, etc.) indicate statistically significant differences between groups ($P < 0.05$) as determined by one-way analysis of the variance with Bonferroni post-hoc test.
IL = interleukin, CON = control, - = Acne group, SE1 = *S. epidermidis* B424F-5, SE2 = *S. epidermidis* BS47C-1, SS = *S. salivarius* BS320F-4, DP = *D. profundi* BS35F-3, COX-2 = cyclooxygenase-2, KC = keratinocyte, iNOS = inducible nitric oxide synthase, TNFα = tumor necrosis factor-α.

day 21, whereas mice treated with SE2, SS, and DP showed visible reductions in these clinical signs (**Fig. 4B**). Histological analysis revealed that the Acne group had dense infiltration of lymphocytes, histiocytes, and neutrophils extending into the dermis and subcutaneous

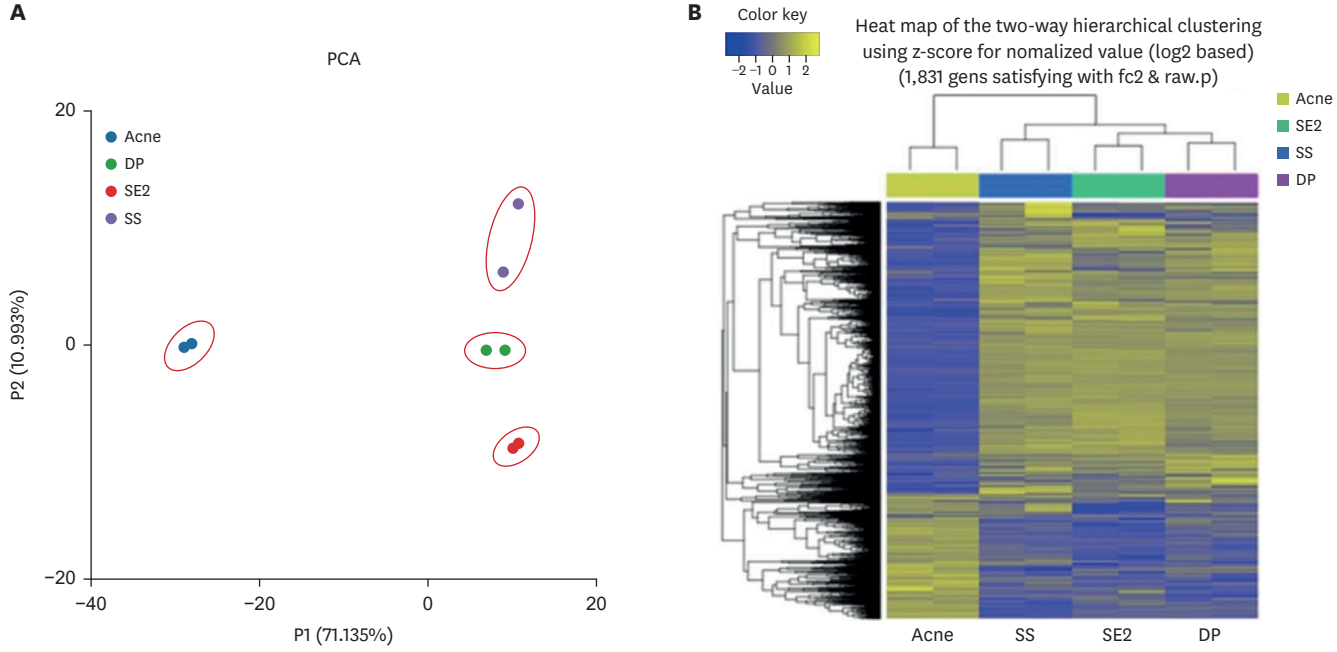

Fig. 3. RNA sequencing analysis of KCs in vitro. (**A**) PCA plot showing distinct clustering by treatment group. (**B**) Heatmap of the top 1,831 differentially expressed genes. (**C**) GSEA results comparing SE2 vs. Acne group. (**D**) GSEA results comparing DP vs. Acne group.
PCA = principal component analysis, SS = *S. salivarius* BS320F-4, DP = *D. profundi* BS35F-3, SE2 = *S. epidermidis* BS47C-1, Acne = only acne treatment group, GSEA = gene set enrichment analysis, KC = keratinocyte.
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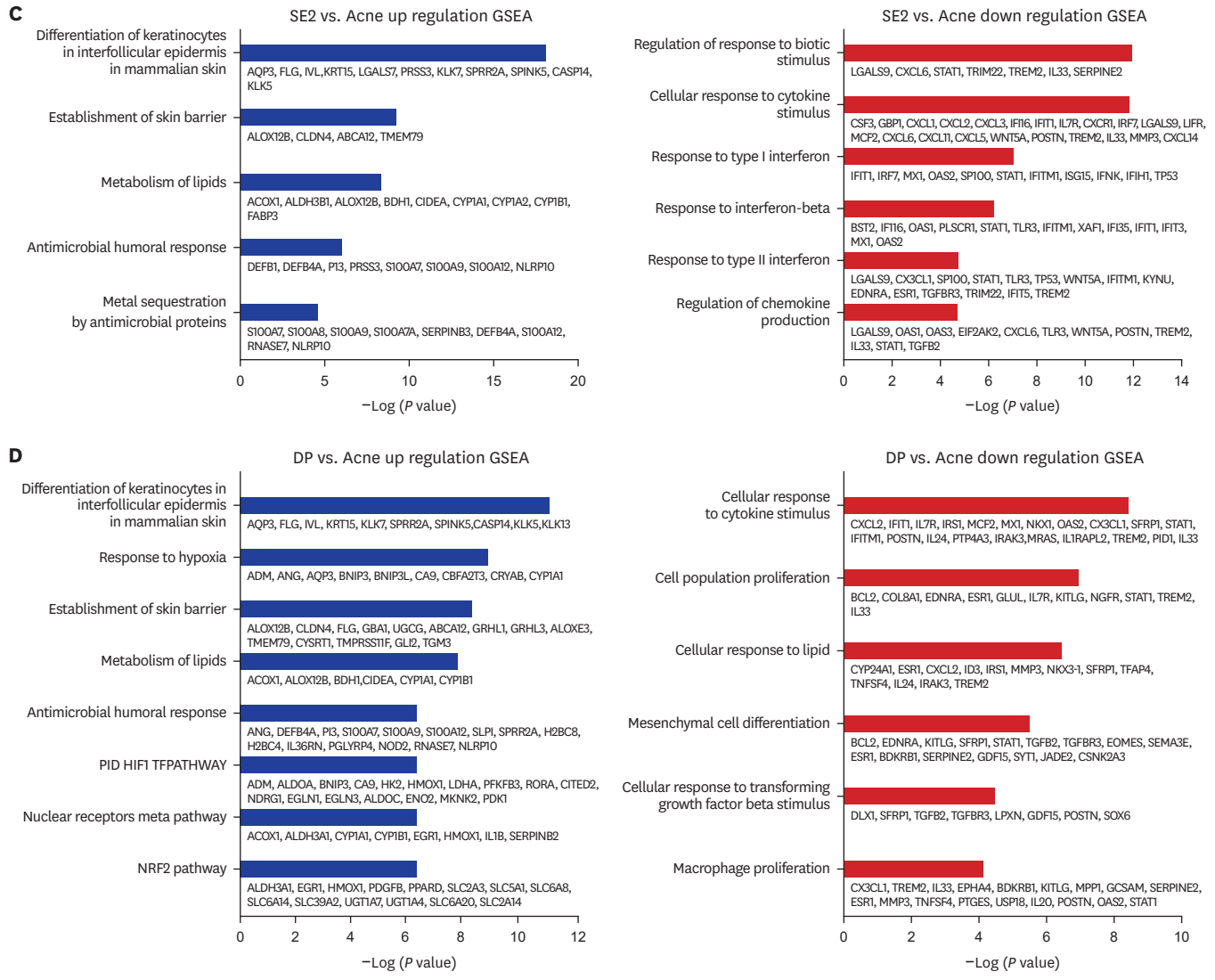

Fig. 3. (Continued) RNA sequencing analysis of KCs in vitro. (**A**) PCA plot showing distinct clustering by treatment group. (**B**) Heatmap of the top 1,831 differentially expressed genes. (**C**) GSEA results comparing SE2 vs. Acne group. (**D**) GSEA results comparing DP vs. Acne group.
PCA = principal component analysis, SS = *S. salivarius* BS320F-4, DP = *D. profundi* BS35F-3, SE2 = *S. epidermidis* BS47C-1, Acne = only acne treatment group, GSEA = gene set enrichment analysis, KC = keratinocyte.

fat (**Fig. 5A**). In contrast, SE2, SS, and DP treatments notably reduced inflammatory cell infiltration and dermal thickening (**Fig. 5F**).

In vivo inflammatory marker expression

qRT-PCR analysis of skin tissue demonstrated significant upregulation of *Il1β, Il8, Tnf-α, inos*, and *Cox2* in the Acne group relative to controls. Topical application of SE2, SS, and DP significantly reduced the expression of these acne-induced inflammatory markers, confirming their anti-inflammatory effects (**Fig. 5G**).

Immunohistochemical analysis

Immunohistochemistry for Caspase-1, IL-1β, and NF-κB further confirmed the inflammatory state induced by *C. acnes* (**Fig. 6A**). Caspase-1 expression was significantly elevated in the Acne group but attenuated following SE2, SS, and DP treatment, with DP showing the

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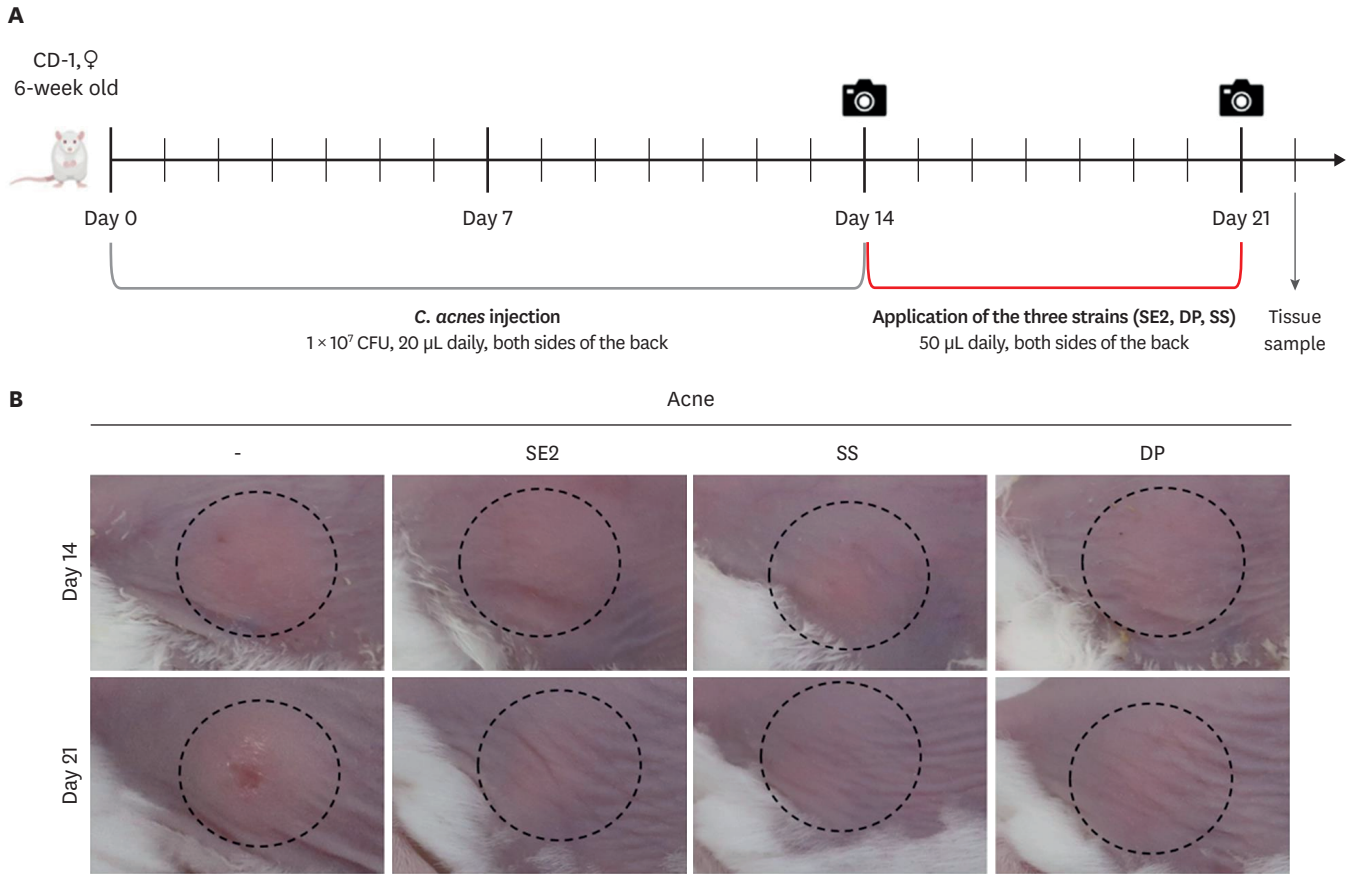

Fig. 4. Schematic of in vivo study design and representative images of *Cutibacterium acnes*-induced lesions. (**A**) Mice received intradermal *C. acnes* injections (1×10^7 CFU/20 μL) daily for 14 days. From Day 14, heat-treated SE2, SS, and DP (1×10^7 CFU/50 μL) were topically applied for 7 days. (**B**) Representative images show persistent erythema in the Acne group on Day 21 vs. Day 14, while SE2, SS, and DP groups displayed reduced swelling and erythema. Dotted circles indicate the treatment area.
- = Acne group, SE2 = *S. epidermidis* BS47C-1, SS = *S. salivarius* BS32OF-4, DP = *D. profundi* BS35F-3.

strongest effect (**Fig. 6B**). IL-1β levels were similarly reduced in all treatment groups, although differences between strains were not statistically significant (**Fig. 6C**). NF-κB expression was markedly increased in the Acne group and significantly decreased by SE2 and SS treatments, with DP showing a moderate reduction (**Fig. 6D**).

DISCUSSION

Acne vulgaris treatment primarily aims to mitigate the inflammatory response induced by *C. acnes* overgrowth, a key factor in acne pathogenesis.[9] Conventional therapies include antibiotics such as clindamycin for their antimicrobial effects,[12] as well as oral isotretinoin and topical retinoids, which reduce sebum production, prevent follicular occlusion, and exert anti-inflammatory actions. Although these treatments are generally effective, they are associated with various side effects such as erythema, scaling, dryness, burning, and pruritus. More importantly, the long-term use of oral or topical antibiotics raises significant concerns regarding the development of antibiotic resistance.[13,14]

In our previous work, we identified four skin microbiome-derived strains—SE1, SE2, SS, and DP—that primarily demonstrated antimicrobial activity against *C. acnes*.[7] In the present

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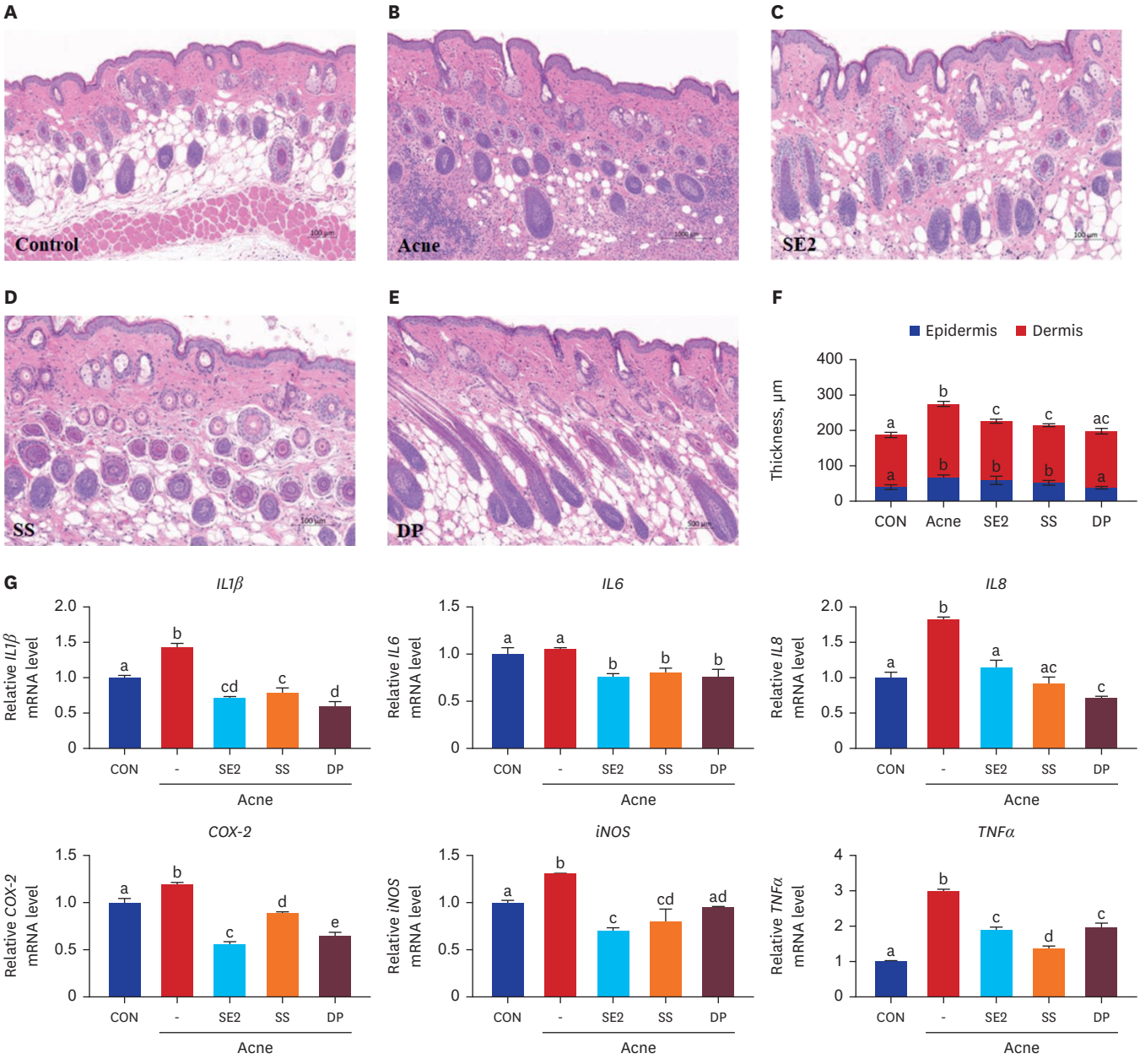

Fig. 5. Histopathology and qRT-PCR of inflammatory markers in vivo. (**A**-**E**) Representative hematoxylin and eosin-stained skin sections. The Acne group showed marked infiltration of lymphocytes, histiocytes, and neutrophils, which was reduced in SE2, SS, and DP groups. (**F**) Epidermal and dermal thickness were significantly decreased in treated groups. (**G**) qRT-PCR showed elevated *Il1β*, *Il8*, *Tnf-α*, *iNOS*, and *Cox-2* in the Acne group, which were reduced following treatment. Data are mean ± SD. Different lowercase letters (a, b, c, etc.) indicate statistically significant differences between groups ($P < 0.05$) as determined by one-way analysis of the variance with Bonferroni post-hoc test (scale bar = 100 μm).
CON = control, - = Acne group, SE2 = *S. epidermidis* BS47C-1, SS = *S. salivarius* BS320F-4, DP = *D. profundi* BS35F-3, IL = interleukin, COX-2 = cyclooxygenase-2, iNOS = inducible nitric oxide synthase, TNFα = tumor necrosis factor-α, qRT-PCR = quantitative reverse transcription-polymerase chain reaction.

study, we evaluated the therapeutic potential of these strains in attenuating *C. acnes*-induced inflammatory responses in both in vitro and in vivo models. To explore their feasibility as safe and stable candidates for topical application, heat-killed preparations of SE2, SS, and DP were used. As a result, these heat-inactivated strains significantly reduced inflammatory responses in keratinocytes and mouse models, highlighting their potential for development as novel topical therapeutics.

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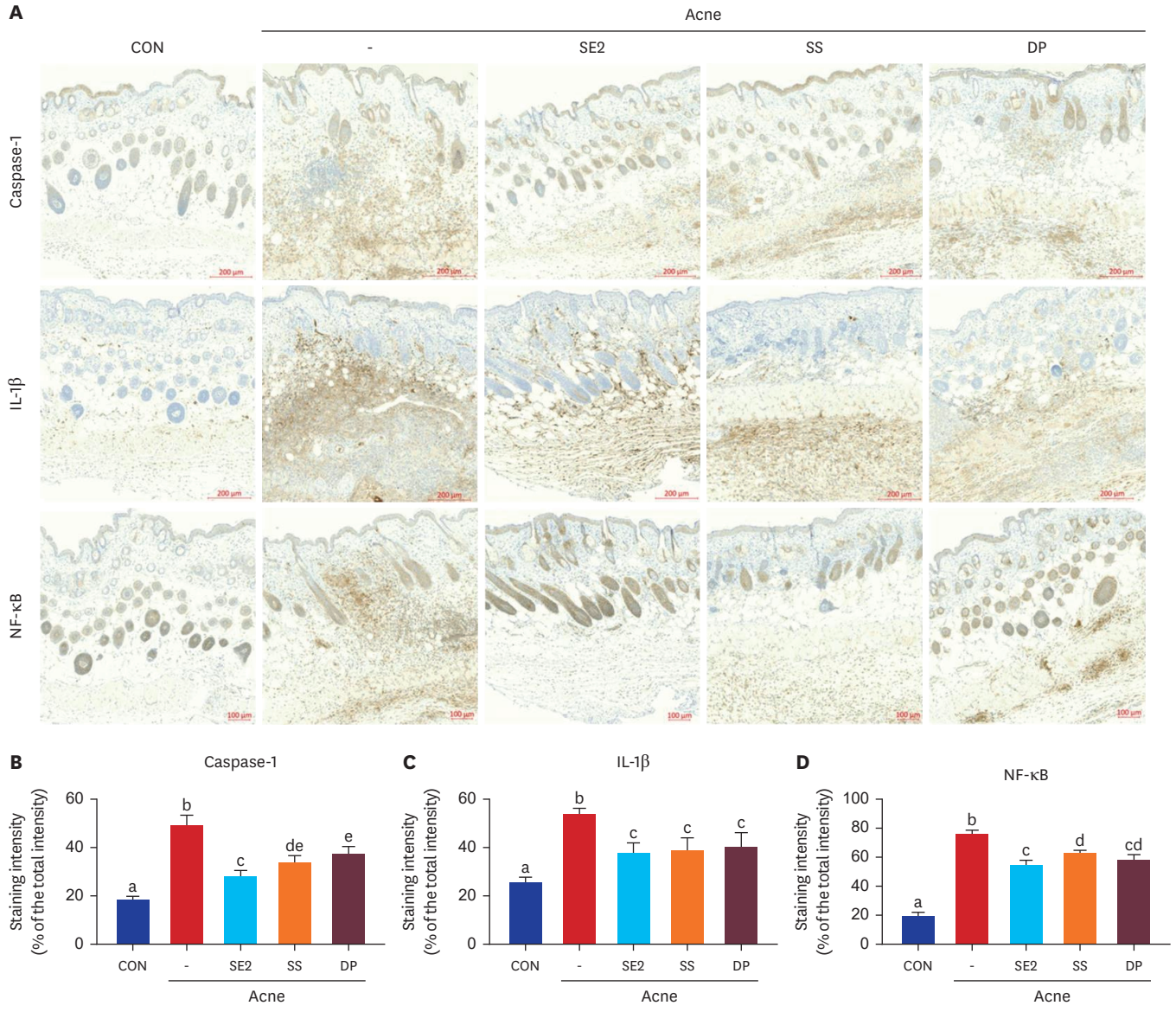

Fig. 6. Immunohistochemical analysis of inflammatory markers in acne-affected skin. (**A**) Representative images showing Caspase-1, NF-κB, and IL-1β staining in skin sections. Brown signals indicate positive expression (scale bar = 200 μm). (**B**-**D**) Quantification of staining intensity for Caspase-1, NF-κB, and IL-1β. SE2, SS, and DP treatments significantly reduced expression levels compared to the Acne group. Data are mean ± SD. Different lowercase letters (a, b, c, etc.) indicate statistically significant differences between groups ($P < 0.05$) as determined by one-way analysis of the variance with Bonferroni post-hoc test.
CON = control, - = Acne group, SE2 = *S. epidermidis* BS47C-1, SS = *S. salivarius* BS320F-4, DP = *D. profundi* BS35F-3, IL = interleukin, NF-κB = nuclear factor kappa-light-chain-enhancer of activated B cells.

In vitro assays revealed that SE2, SS, and DP treatment markedly downregulated acne-related inflammatory mediators, including IL-1β, IL-6, IL-8, COX-2, iNOS, and TNF-α. In vivo, topical application of these strains in a *C. acnes*-induced acne mouse model resulted in visible reductions in swelling and erythema. Histological analysis showed decreased inflammatory cell infiltration and reduced dermal and epidermal thickness in treated groups. These histological improvements were supported by immunohistochemical staining, which demonstrated lower expression of caspase-1, IL-1β, and NF-κB—key markers in acne-associated inflammation.[15-18] RNA sequencing offered further mechanistic insight. Keratinocytes treated with SE2 and DP exhibited upregulation of genes associated with skin barrier function and antimicrobial responses, along with downregulation of cytokine

https://doi.org/10.3346/jkms.2025.40.e327

signaling and interferon pathways, as revealed by GSEA. These transcriptional shifts emphasize the anti-inflammatory and homeostasis-restoring effects of the strains.

Current understanding of acne pathogenesis emphasizes that virulent *C. acne* strains, rather than overall bacterial burden, plays a central role in triggering chronic inflammation, particularly under conditions of skin microbiome dysbiosis.[19] These strains produce a range of virulence factors—including lipases, proteases, hyaluronate lyase, endoglycoceramidases, neuraminidases, and Christie–Atkins–Munch–Petersen factors—that contribute to tissue degradation and promote inflammatory responses.[20-23] These mechanisms facilitate immune cell infiltration into the follicular wall and dermis, aggravating acne lesions.[24] Recent findings further demonstrate that *C. acnes* activates not only classical inflammatory pathways such as NF-κB and the inflammasome but also the type I interferon (IFN-I) signaling axis via the cGAS-STING pathway in human macrophages.[25] Our transcriptomic data showed that treatment with SE2 and DP significantly downregulated interferon-related gene expression. These findings suggest that heat-inactivated commensal strains may attenuate *C. acnes*-induced inflammation not only by counteracting virulence factor-mediated responses but also by suppressing the IFN-I signaling cascade—offering a dual mechanism of therapeutic action.

The potential of probiotic and postbiotic strategies in acne treatment has gained increasing attention. Probiotics exert beneficial effects by inhibiting *C. acnes* through antimicrobial substances, enhancing skin barrier function, and modulating immune responses. For example, *S. salivarius* secretes bacteriocin-like inhibitory substances that suppress *C. acnes* growth,[26] while *Lactococcus* sp. HY449 produces bacteriocins with similar effects.[27] Topical application of probiotics such as *Streptococcus thermophilus* has been shown to enhance ceramide production, thereby reinforcing the skin barrier and contributing to antimicrobial and anti-inflammatory effects.[28-30] Moreover, *S. salivarius* K12 inhibits IL-8 release from keratinocytes,[31] and *Lactobacillus paracasei* NCC2461 reduces substance-induced skin inflammation.[32,33] Our transcriptomic findings align with these reports, as SE2 and DP treatments led to the upregulation of genes related to barrier integrity and innate antimicrobial responses, alongside downregulation of cytokine and interferon-mediated inflammatory pathways. Together, these results support the growing body of evidence that microbiome-derived therapies can modulate acne pathophysiology through both antimicrobial and immunoregulatory mechanisms.

In conclusion, our study demonstrates the efficacy and safety of human skin microbiome-derived, heat-killed strains as potential topical treatments for acne. By modulating both microbial colonization and inflammatory pathways, these strains offer a promising foundation for the development of novel, microbiome-based acne therapeutics. Future clinical studies are needed to confirm their therapeutic potential and safety in human subjects, along with further investigation into their mechanisms of action and optimal formulation strategies. Such efforts will not only validate our findings but also advance the understanding of the skin microbiome's role in acne pathogenesis, paving the way for more targeted and effective treatments.

SUPPLEMENTARY MATERIALS

Supplementary Data 1

Supplementary materials and methods

https://doi.org/10.3346/jkms.2025.40.e327

Supplementary Fig. 1

Cell viability after treatment of *Cutibacterium acnes* on the KCs (**A**) and HDFs (**B**). *C. acnes* was added at five different concentrations (0.25, 0.5, 1, 2, 4 × 10^8 CFU) on KCs (**A**) and HDFs (**B**). Cell viability increased with the increase in CFU of *C. acnes*, which means there was no cytotoxicity of *C. acnes* on both KCs and HDFs.

REFERENCES

1. Habeebuddin M, Karnati RK, Shiroorkar PN, Nagaraja S, Asdaq SMB, Khalid Anwer M, et al. Topical probiotics: more than a skin deep. *Pharmaceutics* 2022;14(3):557. PUBMED | CROSSREF
2. Cruz S, Vecerek N, Elbuluk N. Targeting inflammation in acne: current treatments and future prospects. *Am J Clin Dermatol* 2023;24(5):681-94. PUBMED | CROSSREF
3. Lee YB, Byun EJ, Kim HS. Potential role of the microbiome in acne: a comprehensive review. *J Clin Med* 2019;8(7):987. PUBMED | CROSSREF
4. Myles IA, Moore IN, Castillo CR, Datta SK. Differing virulence of healthy skin commensals in mouse models of infection. *Front Cell Infect Microbiol* 2019;8:451. PUBMED | CROSSREF
5. Chilicka K, Dzieńdziora-Urbińska I, Szyguła R, Asanova B, Nowicka D. Microbiome and probiotics in acne vulgaris-a narrative review. *Life (Basel)* 2022;12(3):422. PUBMED | CROSSREF
6. Dewi DAR, Perdiyana A, Wiliantari NM, Nadhira F, Arkania N, Salsabila CA, et al. Managing the skin microbiome as a new bacteriotherapy for inflammatory atopic dermatitis. *Cureus* 2023;15(11):e48803. PUBMED | CROSSREF
7. Kim JH, Son SM, Park H, Kim BK, Choi IS, Kim H, et al. Taxonomic profiling of skin microbiome and correlation with clinical skin parameters in healthy Koreans. *Sci Rep* 2021;11(1):16269. PUBMED | CROSSREF
8. Li D, Sun Y, Ren X, Zhou N, Li L, He G, et al. Dynamic evaluation of pathological changes in a mouse acne model by optical imaging technology. *Exp Dermatol* 2023;32(9):1350-60. PUBMED | CROSSREF
9. Kolar SL, Tsai CM, Torres J, Fan X, Li H, Liu GY. *Propionibacterium acnes*-induced immunopathology correlates with health and disease association. *JCI Insight* 2019;4(5):e124687. PUBMED | CROSSREF
10. Tsukahara K, Tamatsu Y, Sugawara Y, Shimada K. The relationship between wrinkle depth and dermal thickness in the forehead and lateral canthal region. *Arch Dermatol* 2011;147(7):822-8. PUBMED | CROSSREF
11. Oltulu P, Ince B, Kokbudak N, Findik S, Kilinc F. Measurement of epidermis, dermis, and total skin thicknesses from six different body regions with a new ethical histometric technique. *Turk J Plast Surg* 2018;26(2):56-61. CROSSREF
12. Thiboutot D, Gollnick H, Bettoli V, Dréno B, Kang S, Leyden JJ, et al. New insights into the management of acne: an update from the Global Alliance to Improve Outcomes in Acne group. *J Am Acad Dermatol* 2009;60(5 Suppl):S1-50. PUBMED | CROSSREF
13. Dessinioti C, Katsambas A. *Propionibacterium acnes* and antimicrobial resistance in acne. *Clin Dermatol* 2017;35(2):163-7. PUBMED | CROSSREF
14. Tripathi SV, Gustafson CJ, Huang KE, Feldman SR. Side effects of common acne treatments. *Expert Opin Drug Saf* 2013;12(1):39-51. PUBMED | CROSSREF
15. Qin M, Pirouz A, Kim MH, Krutzik SR, Garbán HJ, Kim J. Propionibacterium acnes induces IL-1β secretion via the NLRP3 inflammasome in human monocytes. *J Invest Dermatol* 2014;134(2):381-8. PUBMED | CROSSREF
16. Fang F, Xie Z, Quan J, Wei X, Wang L, Yang L. Baicalin suppresses *Propionibacterium acnes*-induced skin inflammation by downregulating the NF-κB/MAPK signaling pathway and inhibiting activation of NLRP3 inflammasome. *Braz J Med Biol Res* 2020;53(12):e9949. PUBMED | CROSSREF
17. Noh HH, Shin SH, Roh YJ, Moon NJ, Seo SJ, Park KY. Particulate matter increases *Cutibacterium acnes*-induced inflammation in human epidermal keratinocytes via the TLR4/NF-κB pathway. *PLoS One* 2022;17(8):e0268595. PUBMED | CROSSREF
18. Li ZJ, Choi DK, Sohn KC, Seo MS, Lee HE, Lee Y, et al. Propionibacterium acnes activates the NLRP3 inflammasome in human sebocytes. *J Invest Dermatol* 2014;134(11):2747-56. PUBMED | CROSSREF
19. Cong TX, Hao D, Wen X, Li XH, He G, Jiang X. From pathogenesis of acne vulgaris to anti-acne agents. *Arch Dermatol Res* 2019;311(5):337-49. PUBMED | CROSSREF
20. Lomholt HB, Scholz CFP, Brüggemann H, Tettelin H, Kilian M. A comparative study of *Cutibacterium* (*Propionibacterium*) acnes clones from acne patients and healthy controls. *Anaerobe* 2017;47:57-63. PUBMED | CROSSREF

21. Higaki S, Kitagawa T, Kagoura M, Morohashi M, Yamagishi T. Correlation between *Propionibacterium acnes* biotypes, lipase activity and rash degree in acne patients. *J Dermatol* 2000;27(8):519-22. PUBMED | CROSSREF
22. Holland C, Mak TN, Zimny-Arndt U, Schmid M, Meyer TF, Jungblut PR, et al. Proteomic identification of secreted proteins of *Propionibacterium acnes*. *BMC Microbiol* 2010;10(1):230. PUBMED | CROSSREF
23. Makris G, Wright JD, Ingham E, Holland KT. The hyaluronate lyase of *Staphylococcus aureus* - a virulence factor? *Microbiology* 2004;150(Pt 6):2005-13. PUBMED | CROSSREF
24. Mayslich C, Grange PA, Dupin N. *Cutibacterium acnes* as an opportunistic pathogen: an update of its virulence-associated factors. *Microorganisms* 2021;9(2):303. PUBMED | CROSSREF
25. Fischer K, Tschismarov R, Pilz A, Straubinger S, Carotta S, McDowell A, et al. *Cutibacterium acnes* infection induces type I interferon synthesis through the cGAS-STING pathway. *Front Immunol* 2020;11:571334. PUBMED | CROSSREF
26. Bowe WP, Filip JC, DiRienzo JM, Volgina A, Margolis DJ. Inhibition of *Propionibacterium acnes* by bacteriocin-like inhibitory substances (BLIS) produced by *Streptococcus salivarius*. *J Drugs Dermatol* 2006;5(9):868-70. PUBMED
27. Oh S, Kim SH, Ko Y, Sim JH, Kim KS, Lee SH, et al. Effect of bacteriocin produced by *Lactococcus* sp. HY 449 on skin-inflammatory bacteria. *Food Chem Toxicol* 2006;44(8):1184-90. PUBMED | CROSSREF
28. Di Marzio L, Centi C, Cinque B, Masci S, Giuliani M, Arcieri A, et al. Effect of the lactic acid bacterium *Streptococcus thermophilus* on stratum corneum ceramide levels and signs and symptoms of atopic dermatitis patients. *Exp Dermatol* 2003;12(5):615-20. PUBMED | CROSSREF
29. Di Marzio L, Cinque B, Cupelli F, De Simone C, Cifone MG, Giuliani M. Increase of skin-ceramide levels in aged subjects following a short-term topical application of bacterial sphingomyelinase from *Streptococcus thermophilus*. *Int J Immunopathol Pharmacol* 2008;21(1):137-43. PUBMED | CROSSREF
30. Di Marzio L, Cinque B, De Simone C, Cifone MG. Effect of the lactic acid bacterium *Streptococcus thermophilus* on ceramide levels in human keratinocytes in vitro and stratum corneum in vivo. *J Invest Dermatol* 1999;113(1):98-106. PUBMED | CROSSREF
31. Cosseau C, Devine DA, Dullaghan E, Gardy JL, Chikatamarla A, Gellatly S, et al. The commensal *Streptococcus salivarius* K12 downregulates the innate immune responses of human epithelial cells and promotes host-microbe homeostasis. *Infect Immun* 2008;76(9):4163-75. PUBMED | CROSSREF
32. Guéniche A, Bastien P, Ovigne JM, Kermici M, Courchay G, Chevalier V, et al. *Bifidobacterium longum* lysate, a new ingredient for reactive skin. *Exp Dermatol* 2010;19(8):e1-8. PUBMED | CROSSREF
33. Gueniche A, Benyacoub J, Philippe D, Bastien P, Kusy N, Breton L, et al. *Lactobacillus paracasei* CNCM I-2116 (ST11) inhibits substance P-induced skin inflammation and accelerates skin barrier function recovery in vitro. *Eur J Dermatol* 2010;20(6):731-7. PUBMED | CROSSREF