

Mosquito repellency mediated by skin commensal *Bacillus* species via volatile metabolite reprogramming

Chunxiao Li

aedes@126.com

State Key Laboratory of Pathogen and Biosecurity, Beijing Institute of Microbiology and Epidemiology

Zhenyu Gu

School of Basic Medicine Sciences, Characteristic Key Laboratory of Modern Pathogen Biology,
Guizhou Medical University

Heting Gao

State Key Laboratory of Pathogen and Biosecurity, Beijing Institute of Microbiology and Epidemiology

Hongxia Guo

State Key Laboratory of Pathogen and Biosecurity, Beijing Institute of Microbiology and Epidemiology

Zhihua Fan

State Key Laboratory of Pathogen and Biosecurity, Beijing Institute of Microbiology and Epidemiology

Weixian Zhang

State Key Laboratory of Pathogen and Biosecurity, Beijing Institute of Microbiology and Epidemiology

Dan Xing

State Key Laboratory of Pathogenic and Biosecurity

Xinyu Zhou

State Key Laboratory of Pathogen and Biosecurity, Beijing Institute of Microbiology and Epidemiology

Haotian Yu

State Key Laboratory of Pathogen and Biosecurity, Beijing Institute of Microbiology and Epidemiology

<https://orcid.org/0000-0002-4944-5743>

Teng Zhao

State Key Laboratory of Pathogenic and Biosecurity, Beijing Institute of Microbiology and Epidemiology

Jiahong Wu

School of Basic Medicine Sciences, Characteristic Key Laboratory of Modern Pathogen Biology,
Guizhou Medical University

Article

Keywords: Skin microbiome, Mosquito, Repellency, *Bacillus*, *Macaca fascicularis*

Posted Date: February 3rd, 2026

DOI: <https://doi.org/10.21203/rs.3.rs-8704744/v1>

License: © ⓘ This work is licensed under a Creative Commons Attribution 4.0 International License.
[Read Full License](#)

Additional Declarations: There is **NO** Competing Interest.

Version of Record: A version of this preprint was published at Nature Communications on October 19th, 2026. See the published version at <https://doi.org/10.1038/s41467-026-78356-5>.

Abstract

Individual variation in human attractiveness to mosquitoes critically influences vector-borne disease transmission, but the mechanistic basis remains unclear. This study integrated human skin microbiome profiling, non-human primate in situ intervention, and volatilomics to uncover a microbial-mediated mechanism underlying host-specific mosquito repellency. We established that *Bacillus* species were a core microbial signature associated with reduced mosquito attraction in humans. Targeted Intervention of *B. licheniformis* and *B. paralicheniformis* on primate skin was sufficient to significantly reduce mosquito attraction, thereby establishing a causal microbial contribution beyond descriptive correlations. Mechanistic analyses revealed that *Bacillus* enrichment reprogramed the skin volatilome, specifically increasing the abundance of aromatic and fatty acid-derived effectors, such as p-Cresol and decanoic acid, which associated with repellency phenotypes. Together, these findings revealed a conserved role of skin commensals in shaping mosquito host-seeking behavior, providing a molecular basis for microbiome-informed and bio-inspired strategies for mosquito disease control.

Introduction

Mosquitoes, as medically important insects with global distribution, have triggered a public health crisis that has drawn international attention. Beyond direct discomfort from bites, such as skin redness, swelling, and itching, mosquitoes serve as the primary transmission vectors for various life-threatening mosquito-borne diseases, including dengue fever, Zika virus disease, and malaria ^{1, 2, 3, 4}. In the absence of effective vaccines and targeted therapeutics for most highly pathogenic mosquito-borne viruses, repellents have become a critical line of defense to block disease transmission ^{5, 6}. However, traditional plant-based products suffer from excessively short protection periods, while chemically synthesized repellents, despite strong efficacy, generally exhibit mild toxicity and ecological pollution ^{7, 8}. Therefore developing a novel repellent technology is an urgent public health priority.

Mosquito attraction to human hosts exhibits striking inter-individual variation, with some people consistently attracting fewer mosquitos than others under comparable environmental conditions. This heterogeneity has been repeatedly observed in both daily life and laboratory data. Extensive research has confirmed that human body temperature, exhaled carbon dioxide, and skin-surface volatile chemicals are key signals for mosquitoes to locate hosts ^{9, 10}. Among these, individual differences in skin volatile metabolites are the core factor determining mosquito biting preferences ¹¹. Some populations form a natural defense barrier against mosquitoes due to skin odor composition, exhibiting significantly reduced attractiveness to mosquitoes. As the largest human organ, the skin is colonized by a diverse and complex microbial community ^{12, 13}. volatile organic compounds (VOCs) produced by these microorganisms during metabolism can directly reshape the skin odor profile, thereby regulating mosquitoes' host-seeking behavior ^{14, 15}. This discovery highlights the potential of skin microbiota in repelling mosquitoes and provides an important entry point for exploring natural repellent resources.

In recent years, with advances in high-throughput sequencing technologies such as metagenomics and metabolomics, studies on the link between skin microbiota and mosquito attraction have gradually become a research hotspot. However, a critical scientific gap persists in this field: although human body odor differences drive mosquito preferences and skin microbiota metabolism produces volatile compounds, the specific microbial taxa and underlying molecular mechanisms that establish natural mosquito defense remain unclear. Previous studies have mostly been confined to correlation analyses between microbial community structure and mosquito attractiveness^{16, 17, 18}. They have not identified core microbial species with clear repellent functions or their key metabolites, nor have they systematically analyzed the "microbiota-metabolite-mosquito behavior" regulatory chain, making it challenging to establish causal relationships among the three. Additionally, most existing studies use human volunteers or mice as models^{5, 14, 19, 20}. Human studies are susceptible to interference from complex factors such as genetic backgrounds and living habits, making it difficult to achieve accurate control of experimental variables. Although a single mouse model is suitable for compound activity validation, the species-specific differences in its skin microecology limit the translational relevance of research related to skin microbial colonization.

However, whether specific skin-resident microbes can causally and stably confer mosquito repellency through defined metabolic outputs remains largely unresolved. To address these limitations, this study employs an innovative strategy integrating metagenomics, metabolomics, and a targeted dual-model design, which specifically includes a non-human primate (NHP) model and a mouse model with well-defined adaptive division of labor. The NHP model was used to conduct *Bacillus* spp. smearing experiments, which aligns with the translational requirements of skin microbial colonization research, while the mouse model was employed to complete the validation of repellent compounds, ensuring the accuracy of compound activity evaluation. This dual-model design effectively compensates for the shortcomings of a single model, thereby enhancing the reliability and reference value of the research conclusions. This study also seeks to shift the focus from correlation-based research toward exploring tentative causal links among the microbiota-metabolites-behavior. Specifically, we investigated the association between skin microbial structure and mosquito attraction, screened and validated candidate core microbial species with apparent repellent activity, identified their potential key metabolites, and explored related regulatory mechanisms. Experimental evidence suggests that skin-residing *Bacillus* spp. may serve as key "repellent probiotics", which might exert mosquito-defensive effects by remodeling the skin volatile metabolome. This work provides a new perspective for understanding mosquito host selection, identifies promising candidates of natural and safe repellent microorganisms and metabolic targets, and lays a preliminary theoretical foundation for developing microbiota-based natural repellents, offering potential insights into safe, efficient, and sustainable strategies for mosquito-borne disease control.

Materials and Methods

Volunteers and Experimental Materials

Thirty-five healthy volunteers were recruited publicly and strictly screened based on inclusion criteria. Inclusion criteria: aged 22–32 years, no history of skin diseases, no use of antibiotics, hormonal drugs or mosquito repellent products within the past month, no history of allergies to mosquito bites, and regular living habits.

Three healthy adult cynomolgus monkeys (*Macaca fascicularis*) were selected as experimental animals, reared under controlled conditions (temperature: 22–25°C, humidity: 50%-60%) with free access to food and water. Experiments commenced after 1 month of acclimatization. Laboratory-reared susceptible strains of *Aedes aegypti* were used. Female mosquitoes were maintained on 8% sugar water and fasted for 8 hours prior to experiments. Unfed 5-10-day-old female mosquitoes were selected for attractiveness tests.

All volunteers signed informed consent forms. All animal experiments comply with animal ethics. The study was approved by the State key laboratory of pathogen and biosecurity Ethics Committee.

The bacterial strains used were *Bacillus licheniformis* (BNCC336463) and *Bacillus paralicheniformis* (CICC10181). For functional verification, four key VOCs with chromatographic purity ($\geq 98\%$) were used: p-Cresol (CAS: 106-44-5, Cat. No. C85751-100G, Sigma-Aldrich), Decanoic acid (CAS: 334-48-5, Cat. No. C1875-100G, Beijing Woke Biology Technology Co., Ltd.), 2-Chloroethyl benzoate (CAS: 939-55-9, Cat. No. YS121681-25g, Solarbio Science & Technology Co., Ltd.) and N,N-Dibutylformamide (CAS: 761-65-9, Cat. No. A14533-25ml, Beijing Woke Biology Technology Co., Ltd.). All compounds were dissolved and serially diluted to low (1 mg/mL), medium (10 mg/mL), and high (100 mg/mL) concentration working solutions.

Mosquito Attractiveness Test

A single channel olfactometer was used. Volunteers cleaned their forearms, which were then exposed as the odor source. For the control, no any odor source was placed. Thirty unfed female *Ae. aegypti* were released into the device. After stabilization, mosquitoes were allowed to choose freely for 1 hour, after which the number attracted to each capture area was counted. The relative attractiveness rate for each volunteer was calculated. Volunteers were stratified into high-attractiveness (HA) and low-attractiveness (LA) groups based on the statistical significance of their attraction rates relative to the control (Chi-square test; $P < 0.05$ for HA, $P > 0.05$ for LA)

Collection of Volunteer Skin Microbiota Samples and High-Throughput Sequencing

Skin microbiota samples were collected from the forearms and palms of volunteers immediately following the attractiveness assay. To minimize contamination, sampling was conducted near an alcohol lamp. The skin surface was wiped thoroughly for two minutes using a sterile medical swab moistened with ST solution (0.9% NaCl, 0.1% Tween-20), applied with consistent pressure. Each swab was then

transferred to a tube containing 1 mL of sterile PBS, stored at 4°C, and processed within two hours. All collections were performed by a single operator. A negative control (a swab immersed directly in sterile PBS) was included.

Total genomic DNA was extracted using the E.Z.N.A.® Soil DNA Kit. DNA purity and concentration were measured with a NanoDrop2000, and integrity was checked via 1% agarose gel electrophoresis. Due to insufficient DNA yield and quality for direct library preparation, whole-genome amplification was performed using Multiple Displacement Amplification (MDA). Sequencing libraries were constructed from the amplified DNA, which involved fragmentation (ultrasonic shearing to 300–500 bp), end repair, A-tailing, adapter ligation, and PCR amplification. Library quality was assessed on an Agilent 2100 Bioanalyzer, followed by paired-end sequencing (PE150) on an Illumina NovaSeq 6000 platform to generate raw metagenomic data.

Establishment of In Situ Colonization Model on Non Human Primate Skin

To establish baseline skin microecology and metabolomic profiles, samples were collected from multiple sites on cynomolgus monkeys (back, armpit, upper limb, lower limb, and chest) using the same sterile swab method as for human volunteers, with three technical replicates per site. Total DNA was extracted and subjected to full-length 16S rRNA gene sequencing as previously described. Metabolomic profiling was performed using GC-MS and LC-MS platforms. The resulting microbial community structure and metabolite profiles were analyzed to characterize baseline conditions across sites.

β-diversity analysis (Bray-Curtis distance) was further employed to assess the similarity between back skin and other body sites. These baseline data supported the selection of the back as the target area for subsequent in situ colonization experiments with *Bacillus* strains.

Commensal Bacteria Intervention and NHP Skin Microbiome Sequencing

Newly acquired *B. licheniformis* and *B. paralicheniformis* strains were cultured in nutrient broth at 30°C. Glycerol stocks (25%) were prepared and stored at -80°C. For experiments, stocks were revived and grown to log phase in nutrient broth; bacterial cells were harvested and resuspended at 1×10^8 CFU/mL in fresh medium.

The back skin of cynomolgus monkeys was treated daily for three days by topical application of the bacterial suspension using a sterile swab; control animals received nutrient broth only. Attractiveness to *Ae. aegypti* was assessed before treatment (day 1) and on days 2, 5, and 8 post-treatments. Each monkey was placed inside a metal cage (40×45×70 cm) within an acrylic chamber (50×55×80 cm). Fifty female mosquitoes were introduced into a dual port olfactometer connected to the chamber, and the

number attracted to the treatment side was recorded after one hour (five replicates per time point). Mosquito response was quantified using the Relative Population Index (RPI), calculated as: $RPI = (Ta \times Cb) / (Tb \times Ca)$.

where Ta and Ca represent the average mosquito counts in the treatment group and the control group *after treatment*, respectively, and Tb and Cb represent the average mosquito counts in the treatment group and the control group *before treatment*, respectively.

Skin swabs were collected from the treatment area at the same time point using the standardized sterile swab protocol. Total DNA was extracted by the CTAB method, and PCR amplification was performed with universal primers (27F: 5'-GTGCCAGCMGCCGCGGTAA-3', 1492R: 5'-TACGGYTACCTTGTTACGACTT-3'). After verification of amplified products by agarose gel electrophoresis, single-molecule real-time sequencing was performed on the Pacbio Sequel II platform to analyze dynamic changes in skin microbial community structure before and after treatment.

NHP Skin Metabolomics Detection

Skin metabolites were collected from the test area of cynomolgus monkeys before and after behavioral assays using a magnetic stir bar (Twister, Gerstel GmbH & Co. KG, Germany). The bar was rolled 100 times across the skin, then transferred to a vial. Samples were stored at -20°C and analyzed within 72 h.

Prior to analysis, Twisters were conditioned at 260°C for 2 h. For GC-MS, bars were rinsed with warm pure water and placed in desorption tubes. For LC-MS, samples were extracted with 300 µL of 80% methanol, sonicated for 20 min, and centrifuged; the supernatant was transferred to an injection vial.

GC-MS was performed using a Gerstel TDU2/Trace 1310/Q-Exactive system with helium carrier gas (1 mL/min). The oven program was: 40°C (2 min), ramp to 230°C at 4°C/min, hold 5 min. EI ionization at 70 eV was used, with source/transfer line temperatures of 280/250°C. Data were processed with Xcalibur and TraceFinder; compounds were identified using NIST17 and IAS Center Lab databases.

LC-MS analysis employed an Agilent 1290 HPLC coupled to an AB SCIEX 6600 Q-TOF with ESI. Separation was on an HSS T3 column (2.1 × 100 mm, 1.8 µm) with water (A) and acetonitrile (B), both containing 0.1% formic acid. Gradient: 0–2 min (2% B), 2–14 min (2–98% B), 14–17 min (98% B), 17–17.1 min (98 – 2% B), 17.1–20 min (2% B). Flow rate: 350 µL/min; injection volume: 2 µL. Data were processed with MExplorer Ultimate; peaks with MS1 match and QC RSD > 30% were excluded.

Determination of Protection Time of Metabolomic Compounds

Female *Ae. aegypti* (n = 100) were anesthetized with CO₂ and placed in a 25-cm³ cage. Selected metabolite standards (identified from differential monkey skin metabolomics) were dissolved in absolute ethanol and diluted to three concentrations: 100, 10, and 1 mg/mL.

SPF mice were shaved on the back (2×2 cm) and restrained. The shaved area was treated with 200 µL absolute ethanol for 5 min. Mice were then introduced into the mosquito cage; pairs were considered suitable if ≥ 2 landing/biting attempts occurred within 2 min.

For testing, 200 µL of compound solution was applied to the shaved skin. After 5 min, mice were exposed to mosquitoes, and the time to the first landing/biting attempt was recorded as the protection time (observation limit: 6 h). Tests were performed from low to high concentration with four replicates per concentration. Gloves were changed between solutions; mosquitoes and mice were replaced with different compounds.

Bioinformatics Analysis

Raw data quality was assessed using FastQC (v0.11.9) ²¹. Low-quality reads and adapters were trimmed with Trimmomatic (v0.39) ²². Clean reads were aligned to the human reference genome (GRCh38.p13) ²³ with Bowtie2 (v2.4.5) ²⁴, and host sequences were removed using Samtools (v1.15.1) ²⁵. Taxonomic classification was performed with Kraken2 (v2.1.2) ²⁶, and species abundance was estimated with Bracken to generate taxonomic abundance profiles. LefSe ²⁷ was applied to identify differentially abundant taxa between attractiveness groups: the Kruskal–Wallis H test ($\alpha = 0.05$) was used for screening, followed by linear discriminant analysis (LDA score > 3.0).

Quality control was conducted in QIIME2 ²⁸. After filtering low-quality sequences, chimeras were removed using UCHIME ²⁹ with the Gold database. ASVs were clustered at 97% similarity using DADA2 ³⁰ and annotated against the RDP database ³¹. α - and β -diversity were calculated in QIIME2; group differences were tested with ANOSIM. Differentially abundant ASVs were identified using DESeq2 ³².

Peak detection, alignment, and quantification were performed in Progenesis Q1 to generate a metabolite $\times$ sample matrix. Following missing-value imputation and UV scaling, a PLS-DA model was built with the R package *rop/s*, and model quality was assessed using Q^2 and R^2 . Differential metabolites were selected via OPLS-DA (VIP > 1 , $P < 0.05$).

Differential microbes and metabolites were integrated. Spearman correlation was used to compute associations ($|R| > 0.4$, $P < 0.05$). The overall relationship between microbial communities and metabolomes was examined with the Mantel test. A correlation heatmap was generated in R (v4.4.1), and interactions were interpreted in the context of taxonomic and functional annotations.

Data Statistics and Analysis

Data was analyzed using R 4.4.1 and GraphPad Prism 9.0. Continuous variables are presented as mean $\pm$ standard deviation ($\bar{x} \pm s$). Normality was assessed with the Shapiro–Wilk test. Normally distributed data were compared between two groups using the independent samples t-test, and among multiple groups using one-way ANOVA followed by Tukey's post-hoc test. Non-normal data were analyzed with

the Mann-Whitney U test (two groups) or the Kruskal–Wallis H test with Dunn’s post-hoc test (multiple groups).

For mosquito behavioral assays (volunteer and NHP attractiveness tests), differences in mosquito counts were evaluated with the chi-square test. Changes in attractiveness before and after treatment were assessed using the Relative Population Index (RPI) analyzed by paired t-test. Spearman correlation was used to examine associations between differential microbes, metabolites, and attractiveness parameters ($|r| > 0.4$, $P < 0.05$). The overall relationship between microbial community structure and metabolomic profiles was tested with the Mantel test.

All tests were two-tailed, with $P < 0.05$ considered statistically significant. Figures were generated using R 4.4.1 (ggplot2) and GraphPad Prism 9.0, with consistent formatting.

Results

Skin microbiota composition is associated with differential mosquito attraction in humans Mosquito attraction assays were initially conducted on 37 healthy volunteers (Fig. 1A). Individuals were categorized into high- ($n = 22$) and low-attractiveness ($n = 15$) groups using chi-square tests based on significant intra-individual differences between experimental and control arms (Fig. 1B). Validation of this grouping strategy confirmed that the high-attractiveness group not only exhibited significant within-group differences but also attracted significantly higher mosquito counts compared to the low-attractiveness group (Fig. 1B). Subsequent phenotypic analysis, including age, gender, and BMI, revealed no significant differences between the two groups ($P > 0.05$), thereby excluding these factors as confounding variables (Fig. 1C).

Metagenomic sequencing identified the core skin microbiota of volunteers. At the phylum level, the top 9 dominant phyla (Proteobacteria, Firmicutes, etc.) accounted for 99.92% of total relative abundance (Fig. 1D). At the genus level, the top 9 dominant genera (including *Bacillus*, *Cutibacterium*, etc.) constituted 90.03% of relative abundance and were stably detected in all samples (Fig. 1E). Significant differences in skin microbiota structure existed between the two groups. At the phylum level, Proteobacteria was more abundant in the high-attractiveness group (65.49% vs. 27.65% in low-attractiveness group), while Firmicutes were less abundant (10.93% vs. 65.57%). At the genus level, *Bacillus* abundance was significantly lower in the high-attractiveness group (0.57% vs. 62.72%). α -diversity analysis showed significantly higher Shannon and Simpson indices in the high-attractiveness group ($P < 0.05$), indicating superior species richness and evenness (Fig. 1F), suggesting a close association between microbial diversity and mosquito attraction phenotype. LEfSe analysis ($LDA > 3$, $P < 0.05$) identified 10 differential species. *Staphylococcus epidermidis* and other taxa were enriched in the high-attractiveness group, while *Bacillus* (including *B. licheniformis*, *B. paralicheniformis*), Bacillaceae, and *Nitratireductor* sp. OM-1 were enriched in the low-attractiveness group (Fig. 1G). Normalized absolute abundances of these key bacteria showed consistent variation trends with relative abundances ($P < 0.05$) (Fig. 1H).

Collectively, microbiota differences between groups involve both overall diversity and core taxa of Proteobacteria and Firmicutes. These results indicate that homeostasis alterations mediated by specific functional bacteria drive differential mosquito attraction, providing key targets for mechanism exploration.

For subsequent experiments, *Nitratireductor* sp. OM-1 was excluded due to being unclassified. Among the enriched taxa, *Bacillus* species were prioritized for following validation based on their consistent enrichment in low-attractiveness individuals, known colonization capacity, and reported metabolic versatility.

Topical application of *Bacillus* reshapes the skin microenvironment and confers mosquito repellency in a NHP model

To test whether *Bacillus* spp. enrichment is sufficient to causally reduce mosquito attraction, we established an in situ skin colonization model in cynomolgus monkeys. Three cynomolgus monkeys were subjected to baseline microbiota, metabolome, and attraction assessments. *B. licheniformis* and *B. paralicheniformis* were topically applied to dorsal skin, followed by mosquito attraction assays and multi-omics analysis (Fig. 2A, Fig. 2B). Behavioral tests showed significantly reduced *Ae. aegypti* attraction to *Bacillus*-treated skin compared to the medium control group, with RPI values < 1 at all-time points. Significant repellent effects persisted on days 2, 5, and 8 without attenuation (Fig. 2C). Dynamic monitoring of the microenvironment revealed that colonization by the two **Bacillus** strains significantly decreased microbial α -diversity (Fig. 2D). Prior to intervention, sequencing analysis of the baseline state identified 949 bacterial species across 19 phyla. Consistent dominant bacteria composition among monkeys indicated a stable baseline microbiota, where the top 9 dominant phyla (including Firmicutes, Actinobacteria) accounted for 99.96% of abundance, with Firmicutes (> 50% per site) as the core dominant phylum (Fig. 2E). PCoA (Bray-Curtis distance) based on baseline data showed no site-specific clustering of microbial communities, confirming inter-site similarity (Fig. 2F).

Furthermore, colonization significantly altered the relative abundances of original dominant species, specifically *S. epidermidis*, *Acinetobacter chengduensis*, and *Comamonas testosteroni* (Fig. 2G). Spearman correlation analysis showed significant negative correlations between *Bacillus* abundances and mosquito attraction rate, while *S. epidermidis* abundance was positively correlated with attraction rate (Fig. 2H). These results confirm that both *B. licheniformis* and *B. paralicheniformis* are not merely skin microbiota markers of low-attractiveness populations, but genuine functional effectors that can actively regulate the host skin microenvironment and odor profiles by reshaping the skin microbiota structure, thereby exerting a function of defending against mosquito bites.

Multi-omics Integration Reveals *Bacillus*-Driven Metabolic Reprogramming

PLS-DA of monkey skin metabolites showed no significant inter-site variability, with samples closely clustered in score plots (Fig. 3A), confirming dorsal skin metabolites reliably represent systemic profiles. UPLC-MS/MS and GC-MS analysis showed *Bacillus* colonization reshaped skin metabolic profiles. *B. licheniformis* treatment upregulated 67 and downregulated 12 metabolites; *B. paralicheniformis* treatment upregulated 22 and downregulated 4 metabolites, with 13 co-upregulated and 2 co-downregulated metabolites (Fig. 3B).

Pathway enrichment analysis revealed that KEGG pathway enrichment of the differential metabolites screened after *Bacillus* colonization showed significant enrichment in the "Phenylalanine metabolism" and "Fatty acid biosynthesis" pathways (Fig. 3C). Further verification combined with KEGG functional annotation of the *Bacillus* genome showed that a total of 54 metabolic pathways were enriched in its genome, including "Phenylalanine, tyrosine and tryptophan biosynthesis", "Fatty acid metabolism", "Fatty acid biosynthesis", etc. Among them, functional genes related to the above two pathways consistent with differential metabolite enrichment were highly enriched, reflecting the consistency between genomic functions and metabolomic phenotypes (Supplementary Figure S1).

In addition, this study also performed functional enrichment analysis on the originally dominant bacterial species significantly suppressed after *Bacillus* colonization (including *S. epidermidis*, *A. chengduensis*, and *Comamonas testosteroni*) to further explore the impact of *Bacillus*-mediated microbiota remodeling on skin microecological functions. Functional enrichment results showed that the genes of these three bacteria were significantly enriched in 66 metabolic pathways, 45 of which were the same as those enriched in the *Bacillus* genome. There were also 22 specific metabolism-related pathways, mainly including functions related to aromatic compound degradation, and environmental substance transformation-related functions such as amino acid and vitamin metabolism, fatty acid degradation, and sulfur metabolism (Supplementary Figure S2). In contrast, the *Bacillus* genome also had 11 specifically enriched pathways, whose core functions included carbohydrate metabolism, amino acid and vitamin metabolism, lipid metabolism, and antibiotic resistance-related functions (Fig. 3D).

Based on the "pathway-product" association logic and combined with the pathway enrichment results of differential metabolites, this study identified 4 key VOCs downstream of the core pathways and clarified their potential metabolic pathway origins: p-Cresol is derived from the "Aromatic amino acid metabolism" pathway, Decanoic acid from the "Fatty acid biosynthesis" pathway, while 2-Chloroethyl benzoate and N,N-Dibutylformamide may be associated with fatty acid and aromatic compound-related metabolic pathways.

Mantel test confirmed *Bacillus* abundance was significantly associated with lipids, organic acids, and phenylpropanoids ($r > 0.4$, $P < 0.05$) (Fig. 3E), supporting the bacteria-metabolite link.

Figure 3 Metabolomic reprogramming of skin and induced VOCs profiles following *B. spp.* colonization. (A) PLS-DA score plot verifying the consistency of baseline metabolomic profiles among different skin sites in NHP. (B) Differences in skin metabolites of NHPs following treatment with *B. licheniformis* and *B. paralicheniformis*. (C) Functional category enrichment analysis of skin compounds with differential

expression before and after *B. spp.* application in NHPs. (D) Specific enrichment of functional pathways in *B. licheniformis* and *B. paralicheniformis* relative to native dominant skin bacteria (E) Mantel test revealing associations between dominant skin bacterial genera and metabolite categories.

Functional verification of specific repellent metabolites

A murine model was used to verify the actual mosquito repellent activity of the four compounds (Fig. 4A). GC-MS quantitative detection results subsequently confirmed that the abundances of these four compounds in the skin samples of the two *Bacillus* treatment groups were significantly higher than those in the control group (Fig. 4B). Specifically, in the *B. licheniformis* treatment group: p-Cresol was upregulated by 2.15 folds, Decanoic acid by 2.16 folds, N,N-Dibutylformamide by 2.25 folds, and 2-Chloroethyl benzoate by 29.6 folds. In the *B. paralicheniformis* treatment group: p-Cresol was upregulated by 2.93 folds, Decanoic acid by 4.08 folds, N,N-Dibutylformamide by 2.30 folds, and 2-Chloroethyl benzoate by 5.55 folds. These results further verified the causal relationship between core pathway activation and target metabolite accumulation.

Mosquito repellent time of each compound was determined to quantify its biological activity (Fig. 4C). The results showed that the repellent time of the four compounds increased with increasing concentration, showing a significant concentration-dependent effect. Even at low concentration, the repellent time of each compound was significantly higher than that of the solvent control group. These findings directly confirmed that the four compounds can induce a significant mosquito repellent response, consistent with their upregulated abundances in *Bacillus* treatment groups, and further validated their role as core repellent metabolites.

Survival analysis was further performed to accurately calculate the effective protection time of the four compounds (Fig. 4D). The results showed that the survival curves of the four compounds were significantly separated from that of the solvent control group, with repellent proportion decreasing gradually as protection time extended. Among them, Decanoic acid exhibited the slowest decline rate in repellent proportion, maintaining a high repellent effect for a longer duration. In contrast, p-Cresol showed a relatively faster decline in repellent proportion, corresponding to its lower repellent activity compared with the other three compounds. Notably, with the standard requirement for mosquito repellents being 4 hours of effective protection time, the other three compounds (Decanoic acid, N,N-Dibutylformamide, and 2-Chloroethyl benzoate) met this criterion, while p-Cresol did not. This analysis thus provided a precise characterization of the duration and efficacy of repellent activity for each compound.

Discussion

As the human body's outermost protective organ, skin surface odor is a key signal for mosquitoes to locate hosts^{17, 18}. Skin microbiota are primary contributors to skin odor profiles^{21, 22}, and their compositional homeostasis directly shapes odor spectrum characteristics, which correlate with host attractiveness to mosquitoes²³. Thus, maintaining skin microecological homeostasis is pivotal for

enhancing innate mosquito resistance. This study integrated human mosquito attractiveness phenotyping, primate in situ skin colonization experiments, and multi-omics analyses to explore how two commensal *Bacillus* spp. (*B. licheniformis* and *B. paralicheniformis*) regulate host mosquito attractiveness. Our results supported that colonization of these *Bacillus* strains is associated with skin microbiota restructuring and volatile metabolite alterations, accompanied by reduced mosquito attraction. These findings provide a framework for understanding how skin microecology may contribute to host–mosquito interactions, and we propose a working model to explain its possible metabolic regulatory mechanism.

B. licheniformis and *B. paralicheniformis* belong to the *B. subtilis* group^{24, 25}. Studies confirm this group, including *B. subtilis* and *B. licheniformis*, exerts probiotic effects (e.g., inhibiting *Salmonella* infection, maintaining intestinal microbiota homeostasis, alleviating oxidative stress^{26, 27, 28, 29}), with efficacy and safety validated in clinical trials^{30, 31, 32}. Consistent with their reported probiotic properties in other host-associated contexts, our results suggest that these *Bacillus* species can also modulate skin microbial communities. *Bacillus* strains secrete broad-spectrum antimicrobial substances such as gramicidin and polymyxin^{33, 34, 35, 36}, which counteract *Staphylococcus* spp. by disrupting quorum sensing and biofilm formation. Here, *Bacillus* colonization induced an overall shift in skin microbiota structure, reducing abundances of dominant commensals like *S. epidermidis*—a bacterium that enhances mosquito attractiveness^{20, 37}—and *A. chengduensis*. Inhibiting *S. epidermidis* likely represents one contributing pathway for these *Bacillus* spp. to reduce host attractiveness.

Metabolically, *Bacillus* colonization induced significant changes in skin volatile metabolites. Four key VOCs were upregulated by both strains: 2-Chloroethyl benzoate, Decanoic acid, N,N-dibutylformamide, and p-Cresol, with functional validation confirming their direct mosquito repellent activity. p-Cresol derives from aromatic amino acid metabolism^{38, 39}, and Decanoic acid is a fatty acid biosynthesis product^{40, 41, 42}; both are associated with *Culex* spp. Attractiveness^{43, 44, 45}, and our study extends this association to *Aedes* spp.. In contrast, biosynthetic pathways of non-classical metabolites (2-chloroethyl benzoate, N,N-Dibutylformamide) lack clear annotations in skin microbe-host co-metabolism research^{46, 47, 48}. Elucidating whether these compounds arise from indirect microbial transformation, host–microbe interactions, or environmental derivatization will require further investigation..

Functional annotation clarified the competitive advantages of *Bacillus* spp. and their metabolic regulatory mechanisms. Compared with dominant bacteria (*S. epidermidis*, *A. chengduensis*), *B. licheniformis* and *B. paralicheniformis* are enriched in carbohydrate, lipid, and amino acid metabolic pathways. Skin surface carbohydrates and lipids (from sebum secretion) are core carbon sources for microbiota^{49, 50, 51}; we hypothesize these *Bacillus* strains strengthen their ecological niche by enhancing metabolism of these substrates, supporting stable colonization and microbiota restructuring. Specifically, their enriched "Phenylalanine, tyrosine and tryptophan biosynthesis" pathway provides substrates for p-Cresol synthesis, while the "Fatty acid biosynthesis" pathway consistent with Decanoic

acid production. Meanwhile, suppressed bacteria (*S. epidermidis*, *A. chengduensis*) show impaired fatty acid degradation, further promoting Decanoic acid accumulation.

Based on these findings, we propose a working model of a skin microbiota-mediated repellent framework, herein referred to as the "Skin Probiotic Defense". This framework posits that skin commensals like *Bacillus* spp. may contribute to reduced mosquito attraction by reshaping microbial community structure and associated volatile profiles.. This perspective complements the conventional focus on single repellent molecules, emphasizing the skin microenvironment as a microbial community-modulated chemical ecosystem.

Conclusion

In summary, this study identifies specific skin commensal bacteria as active biological sources of mosquito repellency. Through combined analyses of human cohorts, causal microbial application in a NHP model, and integrative multi-omics profiling, we demonstrate that *Bacillus* spp. are consistently associated with reduced mosquito attraction and are sufficient to induce a robust repellent phenotype on the skin. Simultaneously, the association between *Bacillus* abundance, altered metabolic pathways, and repellent-associated volatile compounds supports a plausible microbial-metabolite-behavior framework underlying this effect (Fig. 5). Unlike previous studies focusing primarily on individual VOCs, our findings highlight a microbiota-centered regulatory paradigm, where *Bacillus* colonization reshapes the skin metabolome and thereby modulates host attractiveness to mosquitoes. While the specific effector metabolites and metabolic pathways remain to be fully resolved, our data support an exploratory microbiota-metabolite-behavior framework linking *Bacillus* colonization to repellent-associated volatile profiles. Collectively, these results highlight the potential of a skin microbiota-derived repellent, expanding mosquito control strategies.

Declarations

Data Availability Statement

The metabolomics data, metagenomic sequencing data, and full-length 16S rRNA gene sequencing data reported in this paper have been deposited in the Genome Sequence Archive (GSA) at the National Genomics Data Center (NGDC), China National Center for Bioinformation/Beijing Institute of Genomics, Chinese Academy of Sciences, under the project accession number PRJCA055301 (<https://ngdc.cncb.ac.cn/bioproject/browse/PRJCA055301>).

References

1. Shah PS et al Comparative Flavivirus-Host Protein Interaction Mapping Reveals Mechanisms of Dengue and Zika Virus Pathogenesis. Cell 175, 1931–1945.e1918 (2018).

2. Probst AS et al (2025) In vivo screen of Plasmodium targets for mosquito-based malaria control. *Nature* 643:785–793
3. Lim A et al (2025) The overlapping global distribution of dengue, chikungunya, Zika and yellow fever. *Nat Commun* 16:3418
4. Ni H et al (2024) Epidemiological characteristics and transmission dynamics of dengue fever in China. *Nat Commun* 15:8060
5. Zhang H et al (2022) A volatile from the skin microbiota of flavivirus-infected hosts promotes mosquito attractiveness. *Cell*
6. Cheng G et al (2010) A C-type lectin collaborates with a CD45 phosphatase homolog to facilitate West Nile virus infection of mosquitoes. *Cell* 142:714–725
7. Chen-Hussey V, Behrens R, Logan JG (2014) Assessment of methods used to determine the safety of the topical insect repellent N,N-diethyl-m-toluamide (DEET). *Parasites vectors* 7:173
8. Swale DR, Bloomquist JR (2019) Is DEET a dangerous neurotoxicant? *Pest Manag Sci* 75:2068–2070
9. Zhao J, Chen AQ, Ryu J, Del Mármol J (2024) Structural basis of odor sensing by insect heteromeric odorant receptors. *Sci (New York NY)* 384:1460–1467
10. Herre M et al (2022) Non-canonical odor coding in the mosquito. *Cell* 185:3104–3123e3128
11. De Obaldia ME et al (2022) Differential mosquito attraction to humans is associated with skin-derived carboxylic acid levels. *Cell* 185:4099–4116e4013
12. Flowers L, Grice EA (2020) The Skin Microbiota: Balancing Risk and Reward. *Cell Host Microbe* 28:190–200
13. Eisenstein M (2020) The skin microbiome. *Nature* 588:S209
14. Wang H et al (2025) Pou2f3 Modulates Mosquito Host-Seeking Behavior by Regulating Skin Microbiota and Their Production of Volatiles: Implications for Eco-Friendly Pest Control Strategies. *J Agric Food Chem* 73:16726–16735
15. Lemfack MC et al (2016) Novel volatiles of skin-borne bacteria inhibit the growth of Gram-positive bacteria and affect quorum-sensing controlled phenotypes of Gram-negative bacteria. *Syst Appl Microbiol* 39:503–515
16. Showering A et al (2022) Skin microbiome alters attractiveness to Anopheles mosquitoes. *BMC Microbiol* 22:98
17. Roodt AP, Naudé Y, Stoltz A, Rohwer E (2018) Human skin volatiles: Passive sampling and GC × GC-ToFMS analysis as a tool to investigate the skin microbiome and interactions with anthropophilic mosquito disease vectors. *J Chromatogr B Anal Technol biomedical life Sci* 1097–1098:83–93
18. Coutinho-Abreu IV, Jamshidi O, Raban R, Atabakhsh K, Merriman JA, Akbari OS (2024) Identification of human skin microbiome odorants that manipulate mosquito landing behavior. *Sci Rep* 14:1631
19. Correa R, Coronado LM, Garrido AC, Durant-Archibold AA, Spadafora C (2017) Volatile organic compounds associated with Plasmodium falciparum infection in vitro. *Parasites vectors* 10:215

20. Liu F et al (2023) Engineered Skin Microbiome Reduces Mosquito Attraction to Mice. the preprint server for biology, bioRxiv
21. Penn DJ et al (2007) Individual and gender fingerprints in human body odour. J R Soc Interface 4:331–340
22. Verhulst NO et al (2010) Differential attraction of malaria mosquitoes to volatile blends produced by human skin bacteria. PLoS ONE 5:e15829
23. Lucas-Barbosa D, DeGennaro M, Mathis A, Verhulst NO (2022) Skin bacterial volatiles: propelling the future of vector control. Trends Parasitol 38:15–22
24. Iqbal S et al (2023) Classification and Multifaceted Potential of Secondary Metabolites Produced by *Bacillus subtilis* Group: A Comprehensive Review. Molecules 28
25. Liu S et al (2025) Enhancement of gut barrier integrity by a *Bacillus subtilis* secreted metabolite through the GADD45A-Wnt/ β -catenin pathway. iMeta 4:e70005
26. Hou Q et al (2022) *Bacillus subtilis* programs the differentiation of intestinal secretory lineages to inhibit Salmonella infection. Cell Rep 40:111416
27. Khan A et al (2023) A gluten degrading probiotic *Bacillus subtilis* LZU-GM relieve adverse effect of gluten additive food and balances gut microbiota in mice. Food Res Int (Ottawa Ont) 170:112960
28. Wen C et al (2023) Engineered *Bacillus subtilis* alleviates intestinal oxidative injury through Nrf2-Keap1 pathway in enterotoxigenic Escherichia coli (ETEC) K88-infected piglet. J Zhejiang Univ Sci B 24:496–509
29. Li Y et al (2020) *Bacillus subtilis* RZ001 improves intestinal integrity and alleviates colitis by inhibiting the Notch signalling pathway and activating ATOH-1. Pathogens disease 78
30. Garvey SM, Mah E, Blonquist TM, Kaden VN, Spears JL (2022) The probiotic *Bacillus subtilis* BS50 decreases gastrointestinal symptoms in healthy adults: a randomized, double-blind, placebo-controlled trial. Gut Microbes 14:2122668
31. Wauters L et al (2021) Efficacy and safety of spore-forming probiotics in the treatment of functional dyspepsia: a pilot randomised, double-blind, placebo-controlled trial. lancet Gastroenterol Hepatol 6:784–792
32. de Medeiros ONPS, de Oliveira FHV, de Almeida Zago TS, Bettiol JR (2023) W. *Bacillus subtilis* and *Bacillus licheniformis* promote tomato growth. Brazilian journal of microbiology: [publication of the Brazilian Society for Microbiology] 54, 397–406
33. Kondrashov OV, Akimov SA, Gramicidin A (2024) in Asymmetric Lipid Membr Biomolecules 14
34. Sun HZ, Li Q, Shang W, Qiao B, Xu QM, Cheng JS (2024) Combinatorial metabolic engineering of *Bacillus subtilis* for de novo production of polymyxin B. Metab Eng 83:123–136
35. Youseif SH, Abdel-Fatah HMK, Khalil MS (2022) A new source of bacterial myrosinase isolated from endophytic *Bacillus* sp. NGB-B10, and its relevance in biological control activity. World J Microbiol Biotechnol 38:215

36. Zhao C, Zhao T, Liu Y, Xia X, Li X (2025) Role of ygaD in mediating polymyxin B resistance in *Bacillus subtilis* via efflux mechanisms. *Microb Pathog* 201:107345
37. Kim D, Crippen TL, Jordan HR, Tomberlin JK (2023) Quorum sensing gene regulation in *Staphylococcus epidermidis* reduces the attraction of *Aedes aegypti* (L.) (Diptera: Culicidae). *Front Microbiol* 14:1208241
38. Gryp T, Vanholder R, Vaneechoutte M, Glorieux G (2017) p-Cresyl Sulfate Toxins 9
39. Billing AM et al (2024) Metabolic Communication by SGLT2 Inhibition. *Circulation* 149:860–884
40. Liao Z, Alrosan M, Alu'datt MH, Tan TC (2024) 10-hydroxy decanoic acid, trans-10-hydroxy-2-decanoic acid, and sebacic acid: Source, metabolism, and potential health functionalities and nutraceutical applications. *J Food Sci* 89:3878–3893
41. Tang Y, Chen L, Yang J, Zhang S, Jin J, Wei Y (2024) Gut microbes improve prognosis of *Klebsiella pneumoniae* pulmonary infection through the lung-gut axis. *Front Cell Infect Microbiol* 14:1392376
42. Yu Z, Han H, Muratkhan M, Ma H, Yue F, Lü X (2024) Screening of lactic acid bacteria with the ability to reduce goaty flavor related fatty acids in goat milk. *Int J Food Microbiol* 423:110832
43. Allan SA, Bernier UR, Kline DL (2006) Attraction of mosquitoes to volatiles associated with blood. *J vector ecology: J Soc Vector Ecol* 31:71–78
44. Lacey ES, Cardé RT (2011) Activation, orientation and landing of female *Culex quinquefasciatus* in response to carbon dioxide and odour from human feet: 3-D flight analysis in a wind tunnel. *Med Vet Entomol* 25:94–103
45. Tauxe GM, MacWilliam D, Boyle SM, Guda T, Ray A (2013) Targeting a dual detector of skin and CO₂ to modify mosquito host seeking. *Cell* 155:1365–1379
46. Lima RD, Hajiababi K, Den Ng B, Sood A, Ferreira RBR (2025) Skin-associated commensal microorganisms and their metabolites. *J Appl Microbiol* 136
47. Hou K et al (2022) Microbiota in health and diseases. *Signal Transduct Target therapy* 7:135
48. Boxberger M, Cenizo V, Cassir N, La Scola B (2021) Challenges in exploring and manipulating the human skin microbiome. *Microbiome* 9:125
49. Dormont L, Bessière JM, Cohuet A (2013) Human skin volatiles: a review. *J Chem Ecol* 39:569–578
50. Taylor D, Daulby A, Grimshaw S, James G, Mercer J, Vaziri S (2003) Characterization of the microflora of the human axilla. *Int J Cosmet Sci* 25:137–145
51. Noël F, Piérard-Franchimont C, Piérard GE, Quatresooz P (2012) Sweaty skin, background and assessments. *Int J Dermatol* 51:647–655

Figures

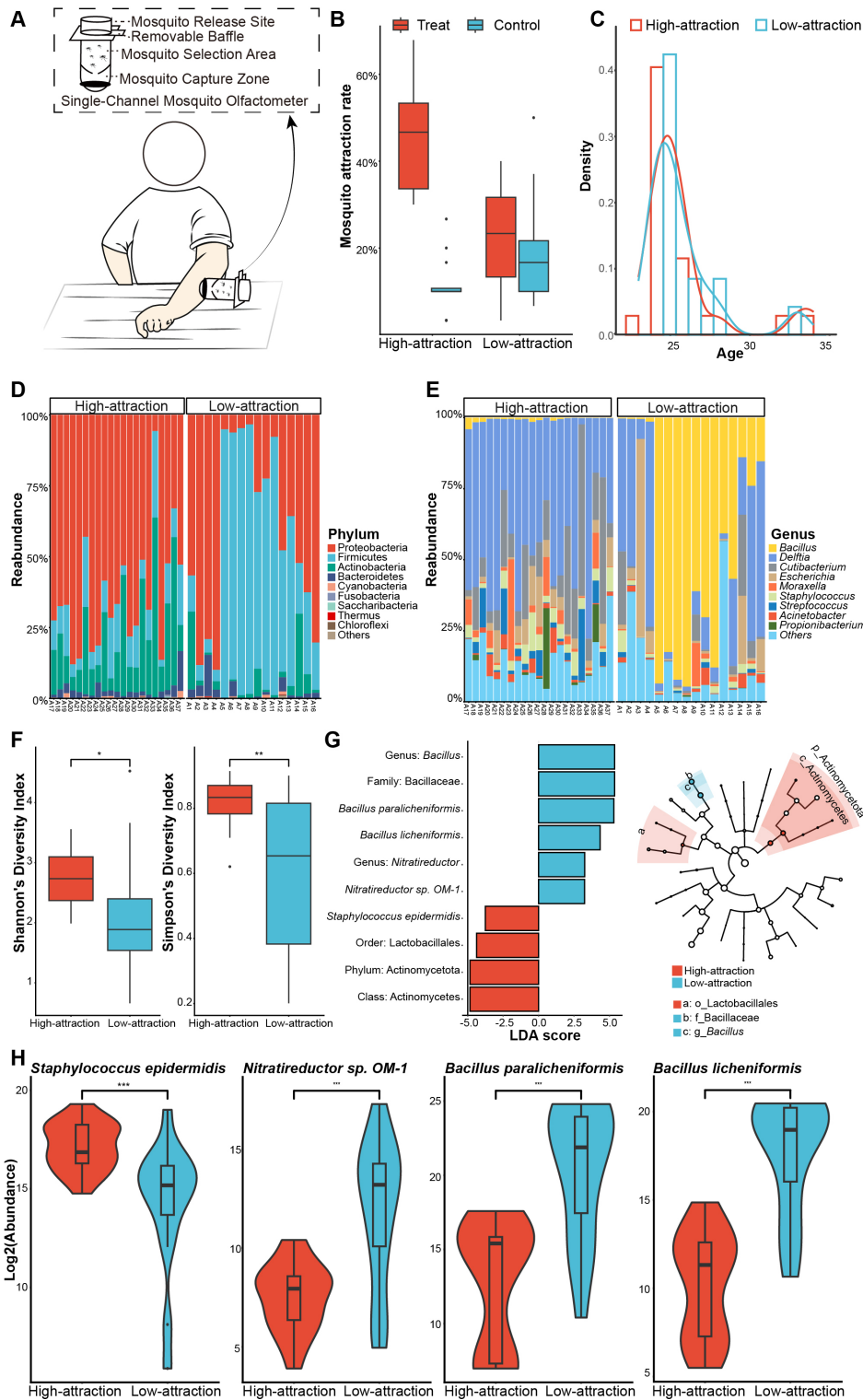


Figure 1

Distinct skin microbiome signatures characterize individuals with innate resistance to mosquitoes in human volunteers. (A) Using a single channel olfactometer to measure the mosquito attraction ability of volunteers. (B) Comparison of mosquito attraction rates between the high-attraction and low-attraction groups. Grouping was determined by single-channel olfactometer assays (Chi-square test, $P < 0.05$). (C) Demographic profiles of volunteers, stratified by mosquito attraction groups. (D) Core dominant

microbial composition at the phylum levels across all volunteers. (E) Core dominant microbial composition at the genus levels across all volunteers. (F) α -diversity indices (Shannon index and Simpson index) of the volunteer skin microbiota across high and low mosquito attraction groups. (G) LEfSe analysis identifying significant biomarkers differentiating LA and HA groups (LDA score > 3, $P < 0.05$). (H) Comparison of absolute abundances for key differential species identified by metagenomic sequencing.

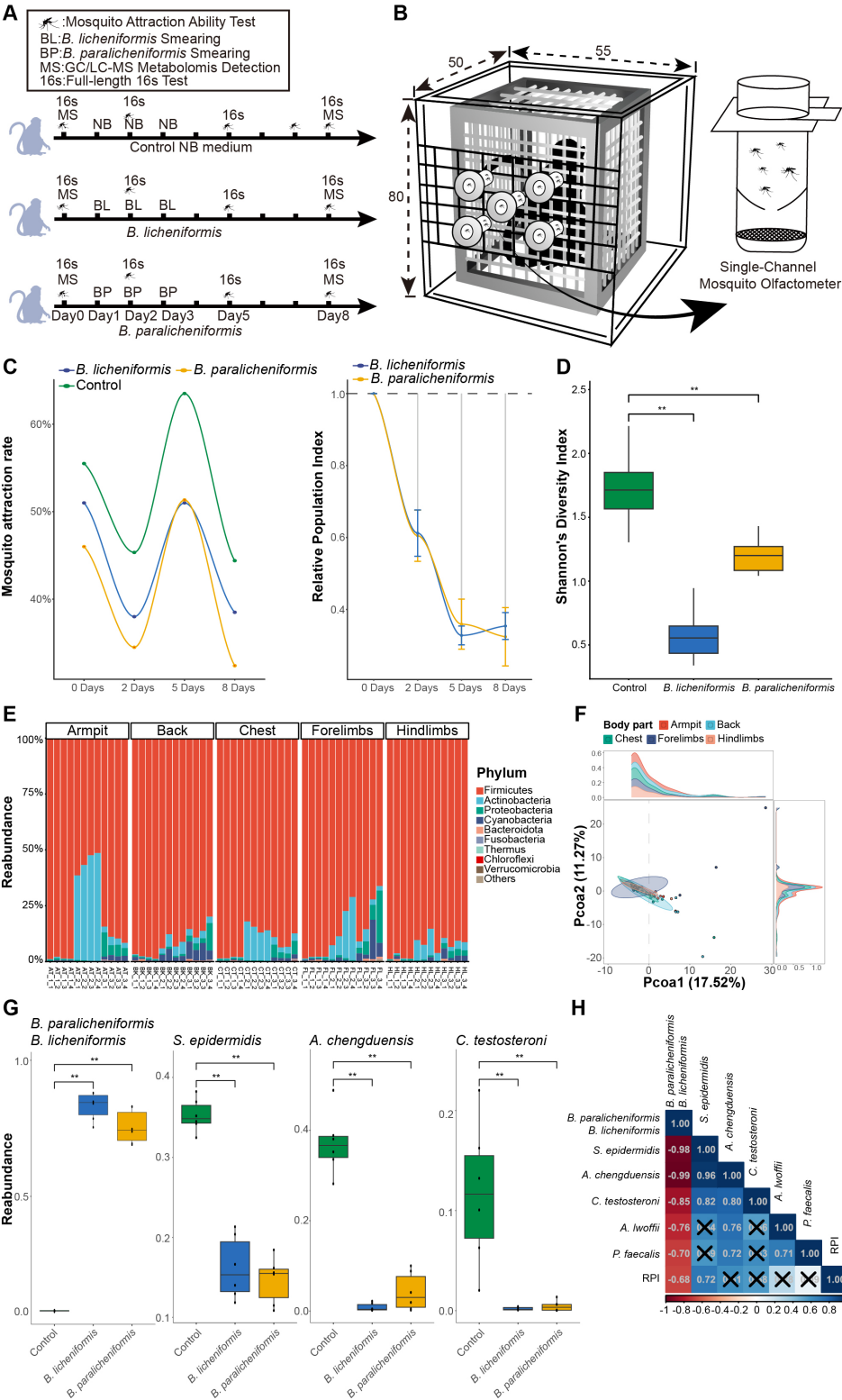


Figure 2

In situ inoculation of *Bacillus* reshapes the NHP skin microbiome and confers mosquito resistance (A) Experimental design for skin bacteria application, microbiome and metabolome detection in NHP (B) Experimental design for mosquito attractiveness of NHP skin (C) Dynamic changes of mosquito attraction and RPI in NHPs across detection time points (D) Changes in alpha-diversity (Shannon index) following the application of *B. spp.* (E) Baseline microbial phylum composition across five distinct body sites of NHPs (n = 3). (F) Pcoa analysis revealing baseline microbiome high similarity across various skin regions of NHPs. (G) Alterations in native dominant skin microbiota of NHP following *B. licheniformis* and *B. paralicheniformis* treatment. (H) Spearman correlation analysis between the abundance of dominant skin bacterial species and mosquito attraction ability.

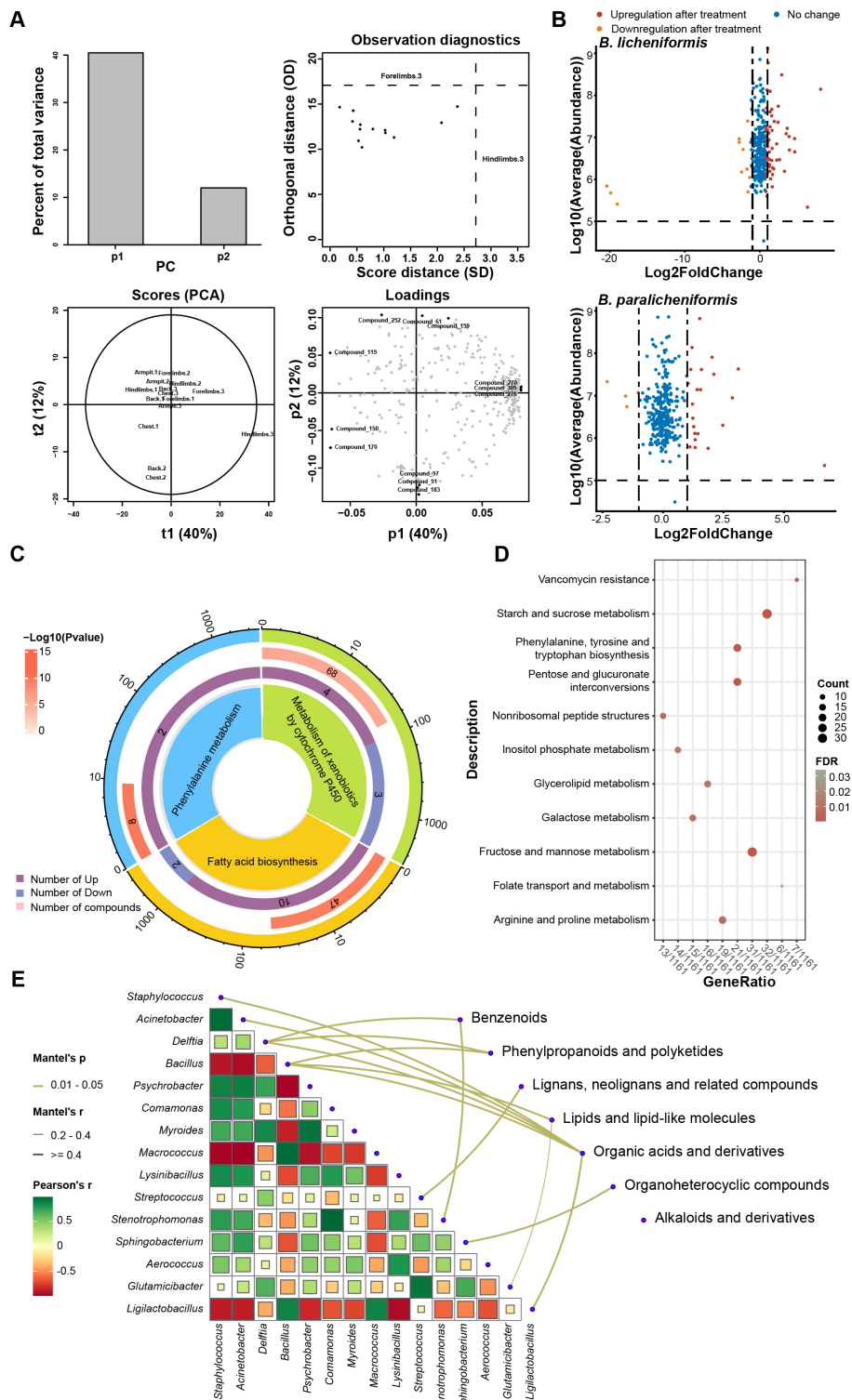


Figure 3

Metabolomic reprogramming of skin and induced VOCs profiles following *B. spp.* colonization. (A) PLS-DA score plot verifying the consistency of baseline metabolomic profiles among different skin sites in NHP. (B) Differences in skin metabolites of NHPs following treatment with *B. licheniformis* and *B. paralicheniformis*. (C) Functional category enrichment analysis of skin compounds with differential expression before and after *B. spp.* application in NHPs. (D) Specific enrichment of functional pathways

in *B. licheniformis* and *B. paralicheniformis* relative to native dominant skin bacteria (E) Mantel test revealing associations between dominant skin bacterial genera and metabolite categories.

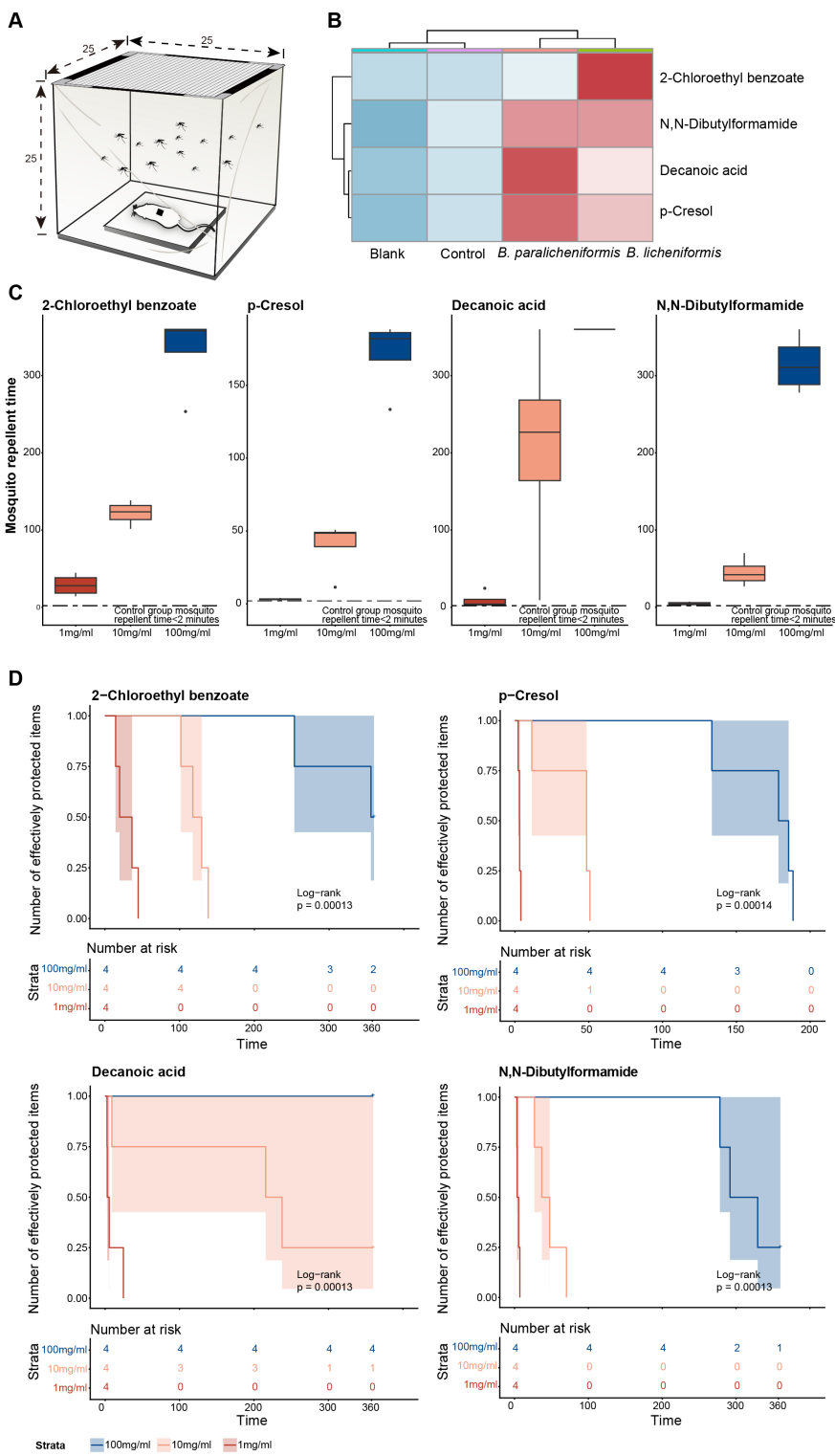


Figure 4

Bacillus-derived metabolites serve as potent olfactory deterrents for host-seeking mosquitoes. (A) Schematic of murine model assay and validation of specific compounds' mosquito repellent duration .

(B) Abundance profiling of the metabolomics-identified specific compound. (C) Dose-dependent distribution of effective protection time for the four compounds. (D) Temporal dynamics of protection failure and time-to-event analysis of breakthrough bites and protected individuals.

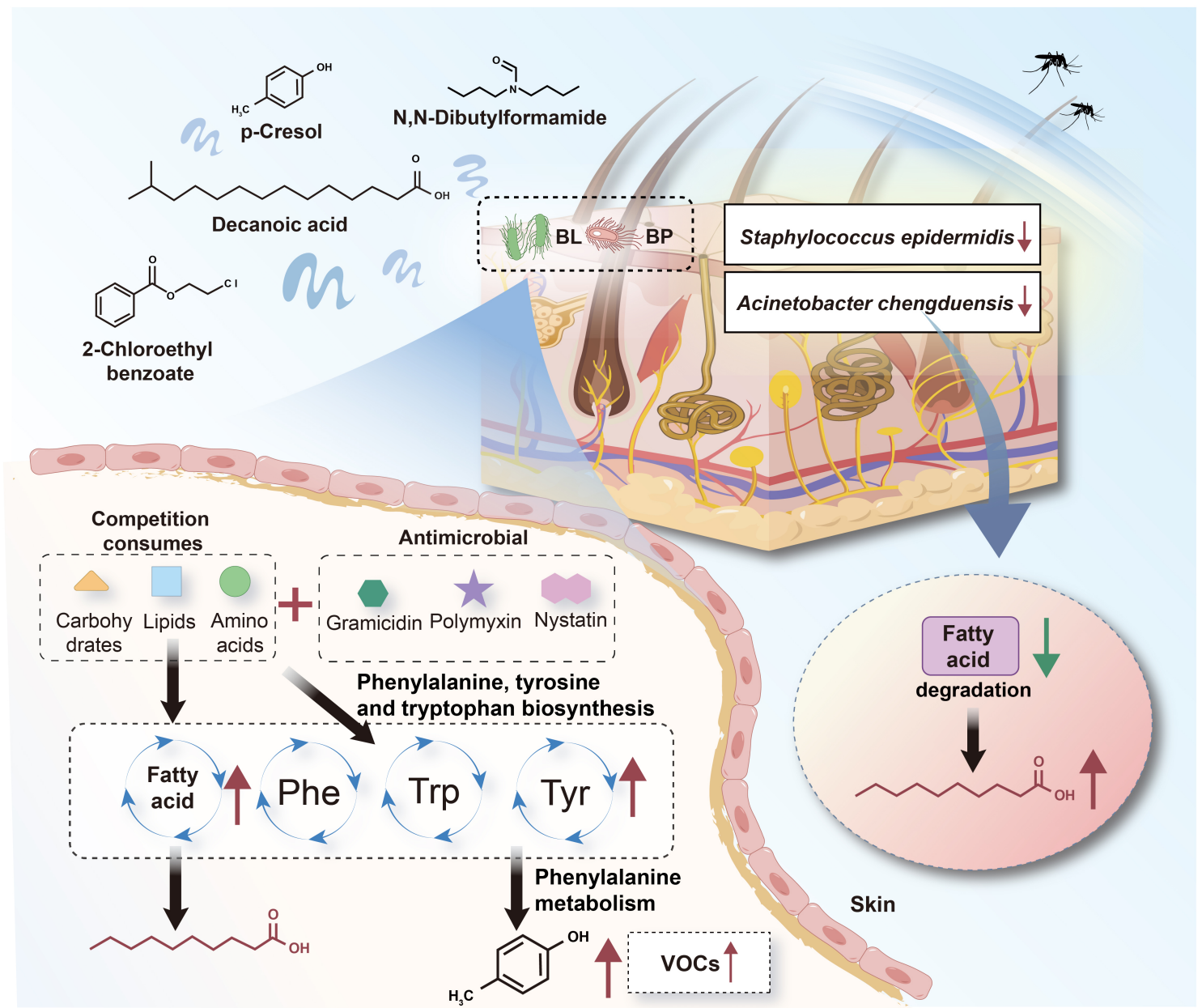


Figure 5

Proposed working model illustrating how *Bacillus* spp. may reshape skin microbiota and volatile profiles to reduce mosquito attraction. On the one hand, it not only provides aromatic amino acid substrates for the repellent substance p-cresol but also directly participates in the synthesis of the repellent substance decanoic acid. On the other hand, the activity of inhibited skin microorganisms (such as *S. epidermidis* and *A. chengduensis*) decreases, leading to a reduction in attractive substances such as small-molecule fatty acids. Ultimately, this achieves the host's protection against mosquitoes.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [SupplementaryFigureS1.jpg](#)
- [SupplementaryFigureS2.jpg](#)