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Enterococcus faecium Kimate-X Mitigates Transport Stress in Dogs via Gut Microbiota Modulation, Elevated SCFAs, and Reduced Cortisol

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ABSTRACT

Stress in dogs during breeding or transport can trigger substantial physiological and behavioral disturbances, including anxiety, appetite reduction, immune suppression, gut microbial imbalance, and even mortality. While pharmacological treatments are widely used for stress management, few specifically target gut health. This study investigates the effectiveness of a new probiotic strain, Enterococcus faecium Kimate-X, in reducing transport-induced stress and supporting intestinal microbiota in dogs, offering a non-drug-based alternative. In vitro experiments revealed that Kimate-X enhanced superoxide dismutase (SOD) and catalase (CAT) activities while decreasing malondialdehyde (MDA) and tumor necrosis factor-α (TNF-α) levels in RAW 264.7 macrophages. In vivo, dogs receiving Kimate-X supplementation exhibited lower cortisol levels after transport, indicating reduced stress. Metagenomic analyses demonstrated increased gut microbial diversity and elevated short-chain fatty acids (acetate, propionate, and butyrate) in feces. These results suggest that Kimate-X alleviates transport stress in dogs by modulating gut microbiota, supporting the use of probiotics as a viable strategy for stress mitigation.

Keywords: Stress, Enterococcus faecium, Gut microbiota, Probiotics, Short-chain fatty acids

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Introduction

Pets have become key members of households in modern society. However, urbanization and lifestyle changes have exposed animals to increasingly complex stressors [1]. Stress is a non-specific physiological reaction that can trigger behavioral alterations and contribute to physiological disturbances such as immune dysfunction, metabolic disruption, and neuroendocrine changes [2, 3]. Despite its prevalence, effective strategies for managing stress in pets remain limited, and owners often lack the tools or knowledge to mitigate it. Among common stressors, transportation is particularly impactful in dogs, as routine travel for veterinary visits, grooming, or relocation can significantly elevate anxiety and physiological stress responses [4].

Pharmacological approaches, including Gamma-Aminobutyric Acid (GABA) agonists (which enhance inhibitory neurotransmission) and Trazodone (a serotonin antagonist), are frequently used to reduce stress in dogs. While effective at reducing anxiety, these drugs may produce side effects such as sedation, behavioral changes, and potential dependency [5]. Therefore, non-pharmacological interventions are increasingly desirable for safe and sustainable stress management.

Recent studies have highlighted the critical role of gut microbiota in the regulation of stress responses [6]. Stress can increase intestinal permeability, promote inflammatory mediator release, and disrupt microbial communities

via activation of the hypothalamic–pituitary–adrenal (HPA) axis and autonomic nervous system [7]. Gut microbial diversity (species richness and abundance) and stability (maintenance of community structure under stress) are key to host health. Diversity enhances functional redundancy and ecological resilience, while stability preserves microbial functions. Disruption of gut microbiota has been observed in both humans and dogs, and maintaining microbial balance is a promising approach to mitigating stress. Probiotics have been shown to restore gut homeostasis, reduce inflammation, and modulate immune responses [8-10].

Probiotics are defined as live microorganisms that provide health benefits when administered in sufficient amounts [11]. In pets, probiotics are widely employed to support digestion and immunity [12-14]. However, research directly linking probiotics to stress mitigation in dogs is limited. Since stress responses involve both hormonal and inflammatory pathways, this study uses transportation as a model of stress and investigates Enterococcus faecium Kimate-X (isolated from healthy police dogs) as an intervention. The study aims to determine whether Kimate-X can modulate stress-related hormone levels, reduce inflammation, and restore gut microbiota balance, thereby clarifying its mechanisms in alleviating transport-induced stress in dogs.

Materials and Methods

In Vitro evaluation of enterococcus faecium Kimate-X on oxidative stress

Mouse RAW 264.7 macrophage cells were maintained in Dulbecco's Modified Eagle Medium (DMEM) enriched with 10% fetal bovine serum (FBS) and 1% antibiotics, incubated at 37 °C with 5% CO_2 in a humidified chamber. Cells at 5×10^5/mL were exposed to 500 ng/mL lipopolysaccharide (LPS) to trigger oxidative stress. At the same time, Enterococcus faecium Kimate-X was added to the culture at a total of 1×10^5 CFU per well (note: CFU per well, not per mL). Three groups were set up for comparison: (1) Control — untreated cells; (2) LPS — cells treated with LPS only; (3) LPS + Kimate-X — cells co-treated with LPS and probiotic to evaluate protective effects. Each experimental condition included three replicates. After 12 hours, supernatants were collected, and levels of SOD, CAT, GSH, MDA, GSH-Px, and TNF-α were measured using ELISA kits (Meilian Biotechnology Co., Ltd., Shanghai, China).

Animal model and experimental setup

Sixteen male Beagle puppies (4–5 months old) were randomly divided into two groups of eight, balanced for body weight (BW). The treatment group received Enterococcus faecium Kimate-X as a freeze-dried powder with skim milk powder as a stabilizer, 1 g/day containing 2×10^9 CFU per dog. The control group received 1 g/day of freeze-dried skim milk without probiotics. Average body weights were 5 ± 0.72 kg for Control and 5 ± 1.21 kg for Kimate-X, with no significant differences ($p > 0.05$). Young male dogs were chosen to reduce variability in immune and stress responses and to minimize sex-based differences.

The study included two phases: feeding (49 days) and transportation (1 day). During feeding, dogs were housed individually in cages (2.5 × 1.5 × 1.5 m), fed at 6:00 AM and 5:00 PM, with free water. No antibiotics were given one month prior to and throughout the study. On day 50, dogs were moved to individual cages for transport, lasting 3 hours over both highways and city roads at ~60 km/h. The compartment temperature and relative humidity were 25 °C and 60%, respectively, starting at 8:00 AM.

All procedures were approved by the Animal Ethics Committee of Nanjing Agricultural University (Approval Number: NJAU. NO20231229197).

Blood sampling and analysis

Immediately post-transport, 3 mL of blood was collected from the forelimb vein using vacuum tubes without anticoagulant. One veterinarian restrained the dog, while another performed the collection. Blood samples were centrifuged at 3000 × g for 10 min at room temperature, and serum was stored at −80 °C. Serum cortisol, SOD, MDA, and TNF-α were measured using commercial ELISA kits (Meilian Bio, Shanghai, China).

Fecal collection and analysis

Fresh feces were collected within 15 minutes of defecation, aliquoted, and stored at −80 °C for subsequent determination of SCFAs and gut microbial composition.

DNA extraction and shotgun metagenomics

Fecal DNA was extracted using the QIAamp PowerFecal Pro DNA Kit (QIAGEN, Hilden, Germany). DNA integrity and contamination were checked on 1% agarose gels, and purity was assessed via 260/280 nm absorbance (OD 1.8–2.0). DNA concentration was quantified with the Qubit™ dsDNA Assay Kit (Thermo Fisher Scientific, Waltham, MA, USA). Samples with OD 260/280 of 1.8–2.0 and ≥1 μg total DNA were used for library construction.

Libraries were prepared from 1 μg of DNA per sample using the NEBNext® Ultra™ DNA Library Prep Kit (NEB, Ipswich, MA, USA) with sample-specific indexes. DNA was fragmented to ~350 bp, end-repaired, A-tailed, ligated to adaptors, and PCR amplified. Purified PCR products were analyzed for size on an Agilent 2100 Bioanalyzer and quantified by qPCR.

Cluster generation was performed on a cBot system, followed by sequencing on an Illumina Novaseq 6000 platform to obtain paired-end reads.

Processing of sequencing data

Adapters and low-quality sequences were removed using Fastp (version 0.23.4, https://github.com/OpenGene/fastp, accessed 16 June 2024). Host-derived reads were excluded by mapping to reference genomes, which were built with bowtie2-build (version 2.5.1, https://github.com/BenLangmead/bowtie2, accessed 16 June 2024), including (1) dog reference genome (Dog10K-Boxer-Tasha, GCA-000002285.5) and (2) human reference genome (GRCh38.p13, NC-000001.11). Unaligned reads were extracted using Samtools (version 1.17, https://github.com/samtools/samtools, accessed 16 June 2024). High-quality, non-host reads were then assembled using MEGAHIT (version 1.2.9, https://github.com/voutcn/megahit, accessed 16 June 2024). Contigs longer than 500 bp were subjected to ORF prediction using Prodigal (version 2.6.3, https://github.com/hyattpd/Prodigal, accessed 16 June 2024). Non-redundant gene catalogs were generated by clustering predicted genes with CD-HIT (version 4.8.1, https://github.com/weizhongli/cdhit, accessed 16 June 2024) at 95% identity and 90% coverage. Gene abundances were quantified using BWA (version 0.7.17-r1198-dirty, https://github.com/lh3/bwa, accessed 16 June 2024) by mapping the high-quality, non-host reads to the non-redundant gene catalog.

Microbial taxonomic and functional profiling

Taxonomic assignment of metagenomic reads was performed with MetaPhlAn4 (https://github.com/biobakery/MetaPhlAn, accessed 16 June 2024) using default settings. Species-level read counts were converted to relative abundances. Gene annotation was carried out with EggNOG mapper (version 2.1.10, https://github.com/eggnogdb/eggnog-mapper, accessed 16 June 2024) based on EggNOG orthologs. Relative abundances for EggNOG genes, KEGG KOs, or pathways were calculated by summing the abundances of genes assigned to the same KOs or pathways.

Alpha (Shannon, Simpson indices) and beta diversity analyses were performed using the vegan and ggplot2 packages in R (version 4.2.1). Differences in alpha diversity were tested with the Wilcoxon rank-sum test. Bray–Curtis distances were used for PCoA to visualize differences in microbial community structure, and significance was assessed with PERMANOVA. LEfSe analysis identified discriminatory taxa between groups with LDA > 2.0. Differential EggNOG KOs and pathways were identified using the same workflow.

Fecal short-chain fatty acid (SCFA) analysis

Fecal samples were analyzed for SCFAs at Zhongke New Life Biotechnology Co., Ltd. (Shanghai, China). Samples were thawed on ice, placed in 2 mL centrifuge tubes, and 50 μL of 20% phosphoric acid was added. 4-Methylvaleric acid (500 μM final concentration) was used as an internal standard. Tubes were mixed for 2 min, centrifuged at 14, 000 × g for 20 min, and supernatants transferred to GC-MS vials. A 1 μL aliquot was injected (split ratio 10:1) into an Agilent DB-FFAP column (30 m × 250 μm × 0.25 μm). Temperature program: 90 °C initial, ramp 10 °C/min to 160 °C, then 40 °C/min to 240 °C, held 5 min. Helium was used as a carrier gas at 1 mL/min. QC samples were inserted periodically to monitor system stability and reproducibility.

Statistical analysis

Normality and variance homogeneity were evaluated using the Shapiro–Wilk and Levene's tests, respectively. For in vitro experiments, one-way ANOVA was applied, followed by Tukey's HSD for multiple comparisons. Non-parametric data were analyzed using Kruskal–Wallis with Dunn's post hoc test. For in vivo data, the Kimate-

X group was compared with the Control group using independent t-tests for normally distributed data or the Mann–Whitney U test for non-normal data. Microbiome diversity indices and community composition differences were assessed using Wilcoxon tests and PERMANOVA (Bray–Curtis). Analyses were conducted in SPSS 26.0, graphs generated in GraphPad Prism 8.0, and microbiome analyses performed in R 4.2.1. Two-tailed tests were used, with $p < 0.05$ considered significant.

Results and Discussion

Kimate-X reduces oxidative stress in RAW 264.7 cells

The protective effects of Enterococcus faecium Kimate-X against LPS-induced oxidative stress were assessed in RAW 264.7 cells. SOD activity in the LPS + Kimate-X group was higher than in the LPS group ($p < 0.01$), but slightly lower than in the Control group **(Figure 1a)**. CAT activity also increased significantly with Kimate-X treatment compared to LPS alone ($p < 0.05$) **(Figure 1b)**. MDA levels in the LPS + Kimate-X group were significantly reduced relative to the LPS group ($p < 0.01$), approaching Control levels **(Figure 1c)**. TNF-α concentrations were markedly lower in the LPS + Kimate-X group than in the LPS group ($p < 0.001$), again nearing Control levels **(Figure 1d)**.

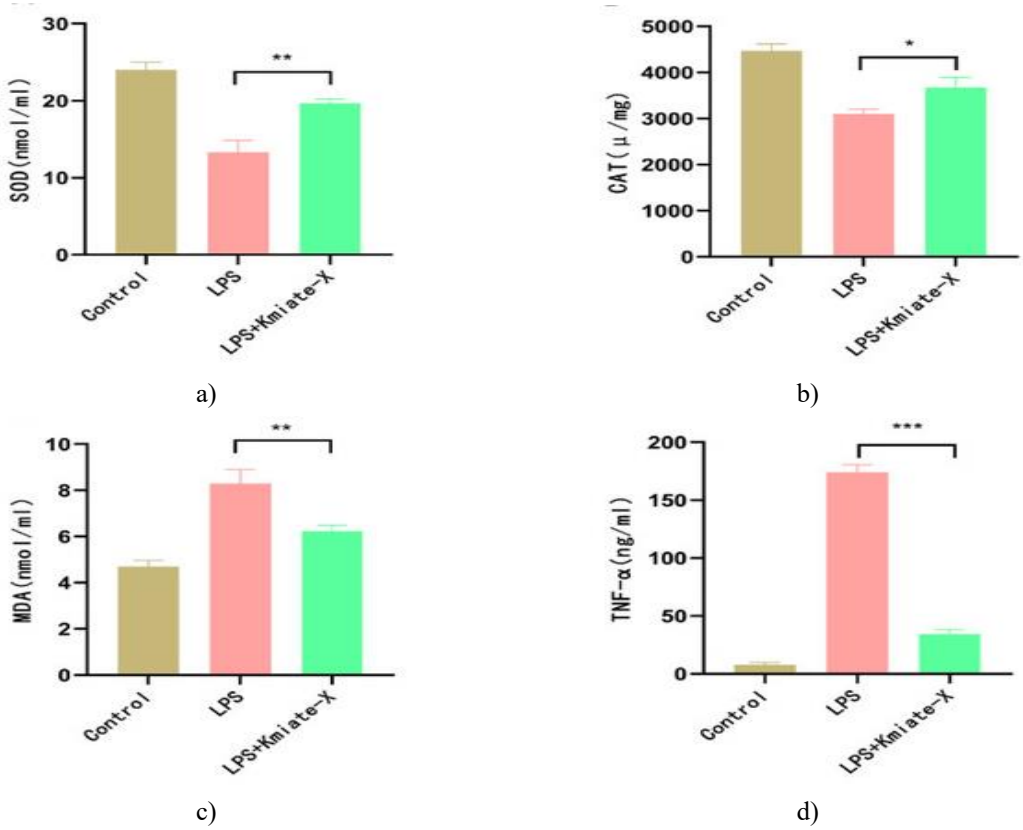


Figure 1. Impact of Enterococcus faecium Kimate-X on oxidative stress induced by LPS in RAW 264.7 cells. Measurements include (a) superoxide dismutase (SOD), (b) catalase (CAT), (c) malondialdehyde (MDA), and (d) tumor necrosis factor-α (TNF-α) in culture supernatants. Data are expressed as mean ± SD from three independent assays (n = 3). Statistical differences were evaluated using Tukey's HSD (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$).

Antioxidant and inflammatory effects of Kimate-X in dogs experiencing transport-induced stress

Based on in vitro findings, the potential of Enterococcus faecium Kimate-X to modulate oxidative and inflammatory responses in transport-stressed dogs was tested **(Figure 2a)**. Post-transport, cortisol levels were lower in the Kimate-X group compared to the Control ($p < 0.05$) **(Figure 2b)**. SOD activity was elevated in Kimate-X-treated dogs ($p < 0.05$) **(Figure 2c)**, whereas CAT activity remained similar between groups **(Figure 2d)**. MDA levels decreased in the Kimate-X group relative to Control ($p < 0.05$) **(Figure 2e)**, suggesting reduced oxidative damage. Inflammatory cytokine TNF-α was also lower in Kimate-X-treated dogs ($p < 0.05$) **(Figure**

2f). Collectively, these results indicate that Kimate-X mitigates physiological stress responses by supporting antioxidant defense and limiting inflammatory activation.

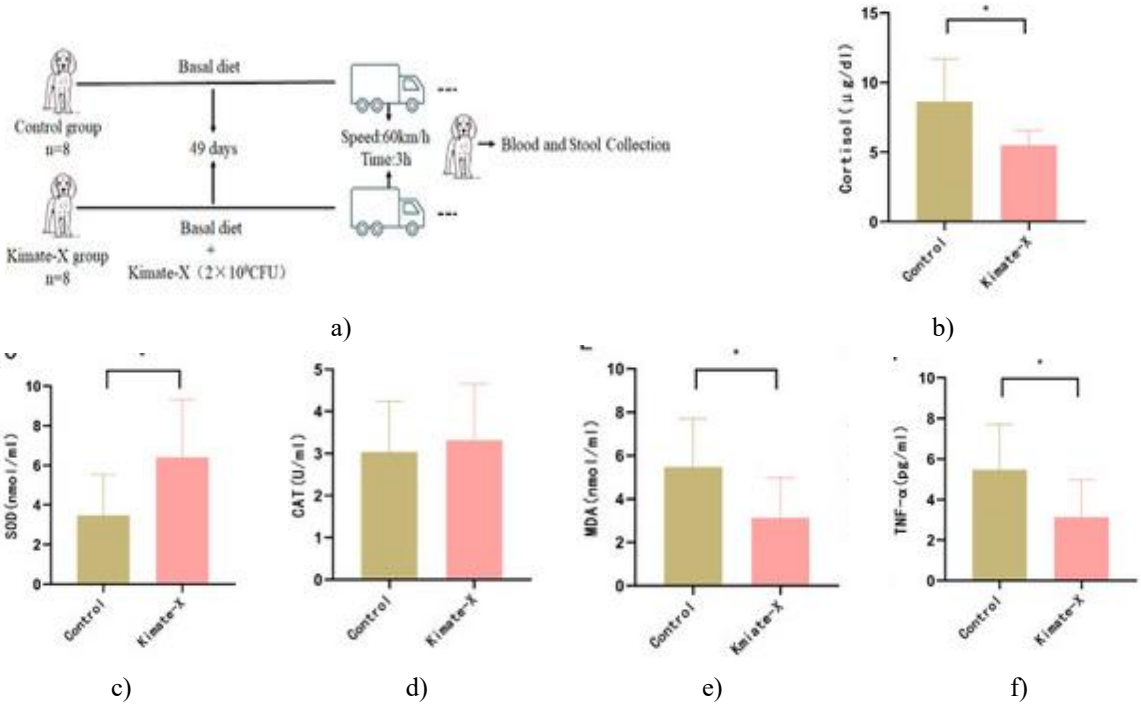


Figure 2. Effects of Kimate-X on oxidative and inflammatory markers in transport-stressed dogs. (a) Experimental workflow; (b) serum cortisol; (c) SOD activity; (d) CAT activity; (e) MDA content; (f) TNF-α levels. Mean ± SD (n = 8 per group). Significant differences were assessed using Student's t-test (* $p < 0.05$).

Fecal microbiome profiling

Metagenomic sequencing was used to examine fecal microbiota in the Control and Kimate-X groups. Alpha diversity indices (Shannon and Simpson) were significantly higher in the Kimate-X group ($p < 0.05$) **(Figure 3a, b)**. PCoA using Bray–Curtis distances revealed clear separation between groups **(Figure 3c)**, reflecting a substantial shift in gut community composition. Bray–Curtis distance comparisons confirmed that the Control group had greater microbial dissimilarity than Kimate-X-treated dogs ($p < 0.001$) **(Figure 3d)**. At the phylum level, overall composition was similar, although relative abundances of major phyla differed **(Figure 3e)**, as visualized in box plots **(Figure 3f)**. Species-level analysis **(Figure 3g)** showed higher Prevotella copri clade A and Streptococcus lutetiensis in Kimate-X dogs, whereas Lactobacillus johnsonii was enriched in Control. Species-level heatmaps **(Figure 3h)** demonstrated that Prevotella copri clade A and Ligilactobacillus animalis predominated in Control, while Streptococcus lutetiensis and Streptococcus alactolyticus were more abundant in Kimate-X-treated dogs, highlighting key taxa associated with Kimate-X intervention.

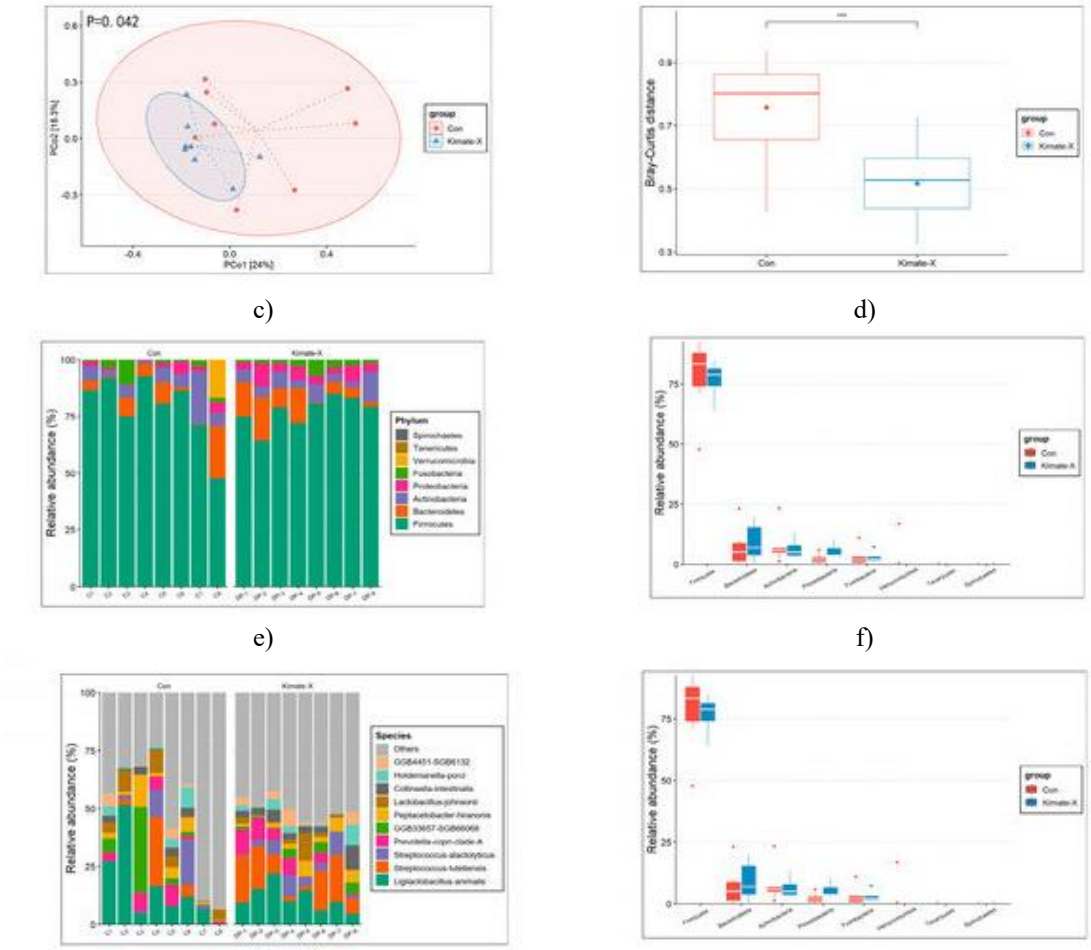
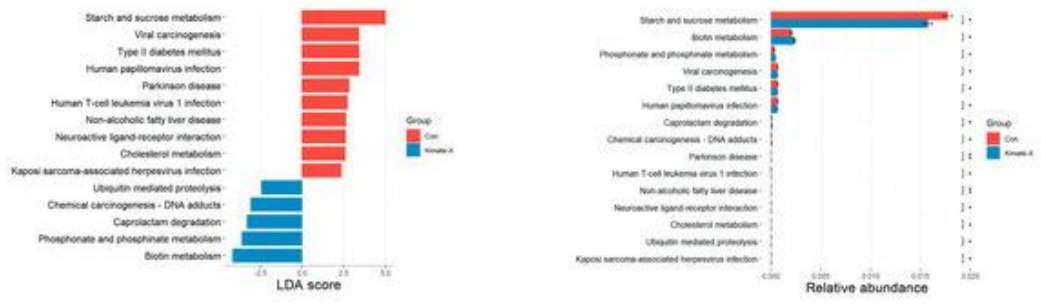
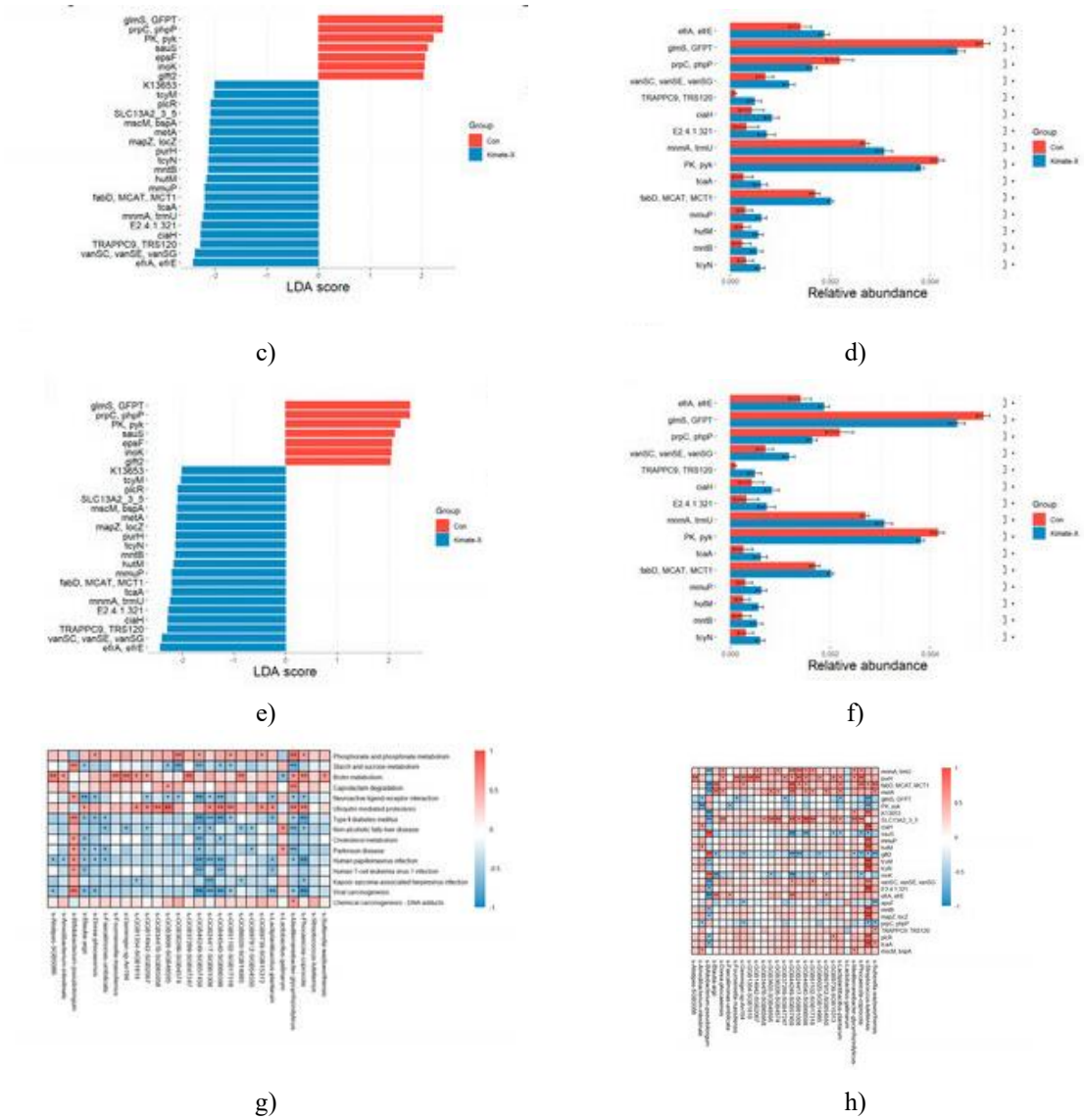


Figure 3. Gut microbiota composition based on metagenomic data. (a) Shannon index; (b) Simpson index; (c) PCoA; (d) Bray–Curtis distance; (e) phylum-level bar plot; (f) phylum-level box plot; (g) selected species-level bar plot; (h) heatmap showing species-level relative abundances (rows = taxa, columns = individual dogs; red = high abundance, blue = low abundance). Statistical significance determined via the Wilcoxon rank-sum test and PERMANOVA (* $p < 0.05$, *** $p < 0.001$).

Functional pathways and gene abundance in gut microbiota

KEGG pathway analysis revealed that starch and sucrose metabolism were enriched in Control, whereas biotin metabolism and phosphate/phosphonate metabolism were higher in Kimate-X-treated dogs **(Figures 4a and 4b)**. KO gene analysis **(Figures 4c and 4d)** showed elevated efrA and efrE in Kimate-X, while glmS and GFPT were enriched in Control. Additionally, vanSC, vanSE, and vanSG genes were significantly more abundant in Kimate-X-treated dogs.





c) d)

e) f)

g) h)

Figure 4. Microbial composition, gene variations, and fecal microbiota associations in dogs

Functional annotations were generated using metagenomic data derived from the same fecal samples described in **Figure 3**. Panels (a, c) illustrate LEfSe-derived linear discriminant analysis (LDA) outcomes identifying pathways or gene functions with significant variation, while panels (b, d) display their proportional abundances. Panel (e) presents a heatmap showing how altered bacterial taxa relate to different metabolic pathways, and panel (f) shows the relationships between species and gene functions. Red shading represents positive relationships, blue represents negative ones, and deeper color tones correspond to stronger association strengths. Statistical significance was determined through LEfSe (Kruskal–Wallis and Wilcoxon tests followed by LDA). Pathways or functions were regarded as significantly different when LDA > 2 and $p < 0.05$ (* for $p < 0.05$; ** for $p < 0.01$).
Analysis of correlations between microbial shifts and metabolic pathways **(Figure 4e)** revealed that starch and sucrose metabolism were positively associated with multiple taxa, notably Bifidobacterium pseudolongum and Lactobacillus gallinarum. Biotin metabolism showed strong positive correlations with Faecalimonas umbilicata and certain unidentified microbes. Additionally, pathways linked to non-alcoholic fatty liver disease and Parkinson's disease demonstrated marked associations with various bacterial species. Correlation mapping between bacterial changes and gene variations **(Figure 4f)** indicated that biotin metabolism-related genes were significantly associated with particular taxa, including Faecalimonas umbilicata. Several unclassified microbes (e.g., GGB3600, SGB4574, GGB3600, and SGB4859) also exhibited notable connections with biotin metabolism pathways.

Fecal short-chain fatty acid (SCFA) analysis

Differences in fecal SCFA concentrations between the Control and Kimate-X groups are summarized in **Figure 5**. Dogs supplemented with Kimate-X showed a pronounced rise in acetate levels compared to Controls ($p < 0.05$). Propionate concentrations were also higher in the Kimate-X group ($p < 0.05$). Likewise, the concentration of butyrate—an essential indicator of intestinal function—was significantly elevated in Kimate-X-treated animals ($p < 0.05$). In contrast, valerate, caproate, and heptanoate levels remained statistically unchanged between the two groups.

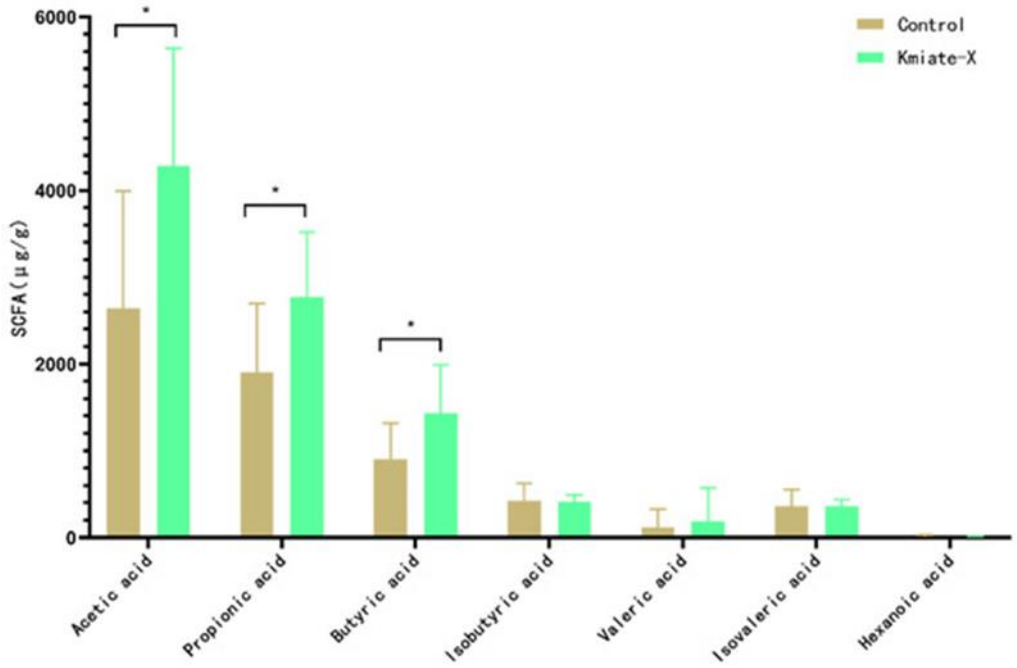


Figure 5. Influence of Enterococcus faecium Kimate-X on fecal SCFA profiles. Fecal samples were assessed via GC–MS for acetate, propionate, butyrate, valerate, caproate, and heptanoate. Data are shown as mean ± SD (n = 8 per group). Significant differences compared with the Control are marked with asterisks ($p < 0.05$, Student's t-test).

Evidence increasingly suggests that stress disrupts intestinal integrity, alters microbial communities, and enhances inflammation, thereby worsening the physiological stress response [15-18]. Concurrently, studies highlight the gut microbiome's essential contribution to stress regulation [19, 20]. The gut–brain axis provides a bidirectional communication network linking the gut to the central nervous system through neural (mainly vagal), hormonal, and immune mechanisms [21]. Through these pathways, intestinal microbes can influence brain activity and host stress physiology by releasing metabolites and signaling molecules such as neurotransmitters.

Certain probiotic strains strengthen intestinal barriers, lower inflammatory responses, generate beneficial metabolites like SCFAs, and modulate neurotransmitter synthesis (e.g., serotonin and GABA), ultimately reducing stress-induced effects [22, 23]. Consequently, probiotic-based therapies are being explored as practical tools to mitigate stress in animals [24]. In this research, we examined the potential of Enterococcus faecium Kimate-X to relieve transport-related stress in dogs and investigated its mode of action.

In cell-based tests, Kimate-X demonstrated strong antioxidative and anti-inflammatory activity. In LPS-stimulated RAW 264.7 macrophages, treatment with Kimate-X significantly enhanced SOD, CAT, and GSH-PX enzyme activities while reducing MDA and TNF-α concentrations compared to untreated controls. These findings imply that Kimate-X strengthens antioxidant systems and suppresses inflammation, consistent with prior reports showing probiotic suppression of NF-κB and MAPK signaling pathways [25]. Such protective effects may also arise from its metabolic products—particularly SCFAs—which are known to counteract oxidative and inflammatory stress [26, 27].

In live-animal experiments, supplementation with Kimate-X reduced stress biomarkers after transport. Dogs receiving the probiotic exhibited elevated SOD and GSH-PX levels and reduced MDA and TNF-α compared to controls, suggesting lower oxidative injury and inflammation. These outcomes agree with previous research

demonstrating probiotic modulation of host stress responses. For instance, Lactobacillus strains have been reported to lower stress-induced cortisol via gut–brain interactions [28]. Similarly, serum cortisol concentrations were significantly lower in the Kimate-X group following transport. Because cortisol serves as a reliable indicator of physiological stress [29], this decrease supports Kimate-X's role in alleviating stress, likely through modulation of the hypothalamic–pituitary–adrenal (HPA) axis, consistent with mechanisms proposed for other probiotics [30].

Gut Microbiota Modulation by Kimate-X

Supplementation with Kimate-X led to marked alterations in the intestinal microbial communities of the dogs. Animals receiving Kimate-X displayed notably greater microbial diversity, reflected in higher Shannon and Simpson index values compared with the Control group, implying that Kimate-X enhanced both species richness and evenness within the gut ecosystem. Elevated diversity is typically linked to stronger intestinal health and greater resilience against stressors [31]. Principal coordinate analysis (PCoA) demonstrated a clear separation between the Kimate-X and Control microbiota profiles, indicating that the probiotic induced substantial restructuring of the gut microbial landscape [32].

At the genus level, several commensal populations shifted in response to Kimate-X. Fiber-degrading taxa such as Prevotella (copri clade A) and Streptococcus (S. lutetiensis) were found in higher relative abundance among treated dogs, whereas Lactobacillus johnsonii appeared more abundant in the Control animals [33]. These compositional changes suggest that Kimate-X fosters an intestinal setting conducive to short-chain fatty acid (SCFA)–producing and beneficial microbes, even when classical probiotic genera like Lactobacillus do not necessarily increase. Such outcomes support the concept that probiotics promote host well-being by reestablishing microbial homeostasis [34].

Functional and Metabolic Shifts in the Microbiome

Metagenomic functional profiling showed that Kimate-X also modified the metabolic potential of the gut community. Dogs supplemented with Kimate-X had greater enrichment of genes involved in biotin metabolism, while Controls exhibited stronger representation of starch and sucrose metabolism pathways. Biotin acts as a vital cofactor in numerous enzymatic reactions, and increased microbial biotin biosynthesis could enhance nutrient availability and intestinal mucosal integrity in the host [35]. Conversely, the relative decline in simple carbohydrate metabolism observed in Kimate-X-fed dogs might indicate a transition toward more efficient energy extraction from complex fibers.

Consistent with these functional changes, fecal measurements revealed that concentrations of acetate, propionate, and butyrate were all significantly higher in the Kimate-X group than in Controls. These SCFAs provide key energy sources for colonocytes and possess anti-inflammatory and immunoregulatory functions; notably, butyrate can inhibit NF-κB activation, thereby reducing intestinal inflammation [36, 37]. Consequently, the ability of Kimate-X to enhance SCFA synthesis and beneficial metabolic activity likely contributes to its stress-protective effects.

Comparison with Conventional Probiotics

Relative to common probiotic genera such as Lactobacillus and Bifidobacterium, Kimate-X exhibits both overlapping and distinctive stress-mitigation mechanisms. Lactobacillus and Bifidobacterium strains are known to alleviate stress by strengthening epithelial barriers, modulating immune pathways, and producing calming neurotransmitters like GABA [38]. Kimate-X appears to operate through similar physiological routes—demonstrated by its reductions in inflammation and cortisol—but does so while reshaping the overall microbial composition in a unique way. Remarkably, Kimate-X elevated the abundance of fermentative and SCFA-generating taxa without necessarily increasing Lactobacillus or Bifidobacterium populations. This pattern suggests that different probiotic species may achieve comparable host outcomes, such as hypothalamic–pituitary–adrenal (HPA) axis modulation and anti-inflammatory effects, via distinct ecological interactions within the microbiota. Additional comparative studies are warranted to evaluate whether Kimate-X's benefits complement or differ from those of traditional probiotics.

Host Variability and Practical Applications

It should be acknowledged that host-to-host variability can influence microbial and physiological responses to probiotic supplementation. In the present work, juvenile male Beagle dogs were selected to reduce confounding factors such as age, sex, and baseline microbial differences, allowing clearer attribution of outcomes to Kimate-X [39, 40]. Nevertheless, variations among breeds, dietary habits, and living environments could modulate probiotic responsiveness in broader dog populations. Future research should address these factors and test Kimate-X in diverse real-world contexts to verify its generalizability and safety.

Despite these considerations, the consistent improvements observed here indicate that Kimate-X holds promise for practical use in promoting canine resilience to stress. The strain could potentially be formulated into pet foods or dietary supplements as a natural aid for managing stressors like travel, confinement, or veterinary handling. Regular administration in veterinary clinics, shelters, or boarding facilities may help reduce stress-induced gastrointestinal disturbances and anxiety, improving overall animal welfare.

Conclusion

In summary, this study provides a scientific basis for translating Enterococcus faecium Kimate-X into applied settings as a novel probiotic candidate for canine stress management. Continued field-based trials are recommended to further confirm its efficacy, safety, and potential integration into daily pet care routines.

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Conflict of Interest: None

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Ethics Statement: None

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