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Microbial community restructuring underlies thermotolerance in heat-stressed lactating sows: An integrated multi-omics analysis

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Introduction

Heat stress (HS) is a critical challenge for livestock production worldwide, projected to intensify with climate change. U.S. losses approach $1 billion annually across the production cycle (Mayorga *et al*., 2019), driven mainly by reduced feed intake, impaired growth, lower reproductive efficiency, and compromised sow performance (Ross *et al*., 2015). Consequences are most severe in lactating animals, which must manage thermoregulation alongside the metabolic demands of milk synthesis. Genetic selection for thermotolerance is a promising long-term resilience strategy. Genomic estimated breeding values (GEBVs) for thermotolerance now exist in multiple species, enabling selection for heat tolerance (Carabaño *et al*., 2017), and moderate heritabilities in lactating sows (h^2 = 0.15–0.29 for vaginal temperature traits) indicate that genetic progress is achievable (Freitas *et al*., 2023). Yet the molecular mechanisms underlying this genetic variation—particularly host–microbiome interactions—remain poorly understood. The gut microbiome encodes metabolic capabilities far beyond the host genome, functioning as a "second genome" that may aid host adaptation (Cho and Blaser, 2012). HS profoundly alters gut microbial composition and function in livestock, with changes tracking metabolic and performance impairments (He *et al*., 2019; Xiong *et al*., 2022). How microbial communities respond to HS in genetically divergent animals, however, has not been characterized using integrated multi-omics. We compared thermotolerant (TOL) and thermosensitive (SEN) lactating sows, divergently selected on GEBVs, during controlled cyclic HS, integrating metagenomics, metatranscriptomics, and metabolomics. Our objectives were to (1) identify temporal patterns of microbial and metabolic adaptation to HS; (2) characterize differential response trajectories between lines; and (3) integrate findings across platforms to identify coordinated mechanisms underlying thermotolerance.

Materials & Methods

Animals and experimental design. Twenty-five multiparous sows (parities 2-5) from a Landrace × Yorkshire population were classified based on GEBVs for thermotolerance (Byrd *et al*., 2025). GEBVs were derived from a population phenotyped for vaginal temperature responses during standardized heat challenges (Wen *et al*., 2023; Usala *et al*., 2021). Animals were ranked by GEBV: 13 sows with favorable GEBVs were designated thermotolerant (TOL) and 12 with unfavorable GEBVs as the SEN group. Following selection, sows were housed individually in climate-controlled chambers at the USDA-ARS Livestock Behavior Research Unit (West

Lafayette, IN, USA). From day 4 of lactation, cyclic HS was imposed: chamber temperatures cycled between 32°C (0800-1700 h) and 28°C (1700-0800 h) with relative humidity at 50-70%, sustained through day 18 of lactation.

Multi-omics profiling. Fecal samples were collected at multiple timepoints for profiling. Metagenomic samples (days 4, 8 and 14) underwent shotgun sequencing, assembly using metaSPAdes within the ATLAS pipeline, and metagenome-assembled genome (MAG) recovery via VAMB binning. MAGs were quality-filtered (>50% completeness, <10% contamination) and taxonomically classified with GTDB-Tk. Metatranscriptomic profiling (days 0 and 18) assessed active gene expression using HUMAnN3, generating KEGG Orthology (KO)-level expression estimates. Metabolomic profiling (days 0, 1, 8, 18) employed untargeted LC-MS/MS on fecal extracts, yielding 12,714 metabolite features.

Guild-based analytical framework. We developed a hierarchical guild system organizing molecular features into functionally related groups based on KO assignments. MAGs were assigned to guilds when ≥50% of core pathway KO terms were detected, ensuring functional classification. This framework captured 20 functional guilds for metagenomics and 22 for metatranscriptomics, enabling direct comparison of functional potential (metagenomics) versus actual activity (metatranscriptomics). Metabolites were organized into 31 Class I and 103 Class II chemical guilds based on chemical taxonomy.

Statistical analyses. Linear mixed-effects models assessed temporal, genetic line, and interaction effects, with animal as random effect. Models were fitted using REML via lme4 in R, with significance testing using Type III F-tests with Satterthwaite's degrees of freedom. Cohen's d effect sizes quantified between-line differences at each timepoint, classified as negligible ($|d| < 0.2$), small (0.2-0.5), moderate (0.5-0.8), or large ($|d| \geq 0.8$). Post-hoc power analysis indicated approximately 60% power for detecting large effects ($d = 0.8$) and 35% for medium effects ($d = 0.5$).

Results & Discussion

Temporal dynamics dominated across all three omics platforms, with timepoint effects explaining substantially more variance than genetic line effects or their interactions. Critically, minimal constitutive differences existed between genetic lines at baseline, but substantial divergence emerged during HS exposure. This pattern suggests that thermotolerance may manifest primarily as differential adaptive capacity rather than fixed metabolic programming.

Metagenomic findings. Effect size analysis revealed large between-line differences ($|d| > 0.8$) emerging specifically at day 14 for five functional guilds (Table 1). Negative Cohen's d values indicated higher abundance in TOL animals. Importantly, effect sizes at day 4 were negligible to small ($|d| < 0.5$) for most guilds, indicating that group differences developed progressively during HS exposure rather than existing constitutively. Methanogens showed the largest effect ($d = -0.920$), with TOL animals exhibiting increased abundance (+12.8%) while SEN animals showed decreased abundance (-15.3%). This divergent pattern suggests that enhanced hindgut methanogenesis may contribute to thermotolerance mechanisms.

Table 1. Metagenomic guild effect sizes (Cohen's d) by timepoint. Negative values indicate higher abundance in TOL animals. Large effects ($|d| \geq 0.8$) shown in bold.

Guild	Day 4	Day 8	Day 14
Methanogens	-0.169	-0.541	-0.920
Fermentation	0.252	-0.346	-0.907
Motility/Chemotaxis	0.418	-0.502	-0.894
Polysaccharide Degradation	0.326	-0.310	-0.836
SCFA Production	0.176	-0.348	-0.812

Metatranscriptomic findings. A striking pattern emerged when comparing transcriptional activity between genetic lines: TOL animals showed widespread transcriptional downregulation in 15 of 22 guilds from day 0 to day 18, while SEN animals showed significant decline in only one guild (Electron Transport). Table 2 presents selected guilds showing the largest differential responses between genetic lines. The most pronounced differences were observed in Biofilm Formation (TOL: -68.5% vs SEN: +42.3%), Carbohydrate Metabolism (TOL: -57.3% vs SEN: +2.7%), and Fermentation (TOL: -51.0% vs SEN: -12.9%).

Table 2. Metatranscriptomic temporal changes (% change from day 0 to day 18) by genetic line. *$p < 0.05$.

Guild	TOL %Δ	SEN %Δ	Δ(TOL-SEN)
Biofilm Formation	-68.5*	+42.3	-110.8
Carbohydrate Metabolism	-57.3*	+2.7	-59.9
Fermentation	-51.0*	-12.9	-38.0
Stress Response	-35.6*	-1.3	-34.3
Methanogens	+48.0	+28.6	+19.4

Capacity-activity discordance. A notable pattern emerged when integrating metagenomic and metatranscriptomic findings: TOL animals showed increased metagenomic capacity (Fermentation: +10.5%; SCFA Production: +15.9%; Methanogens: +12.8%) coupled with decreased metatranscriptomic expression in 15 of 22 guilds. Conversely, SEN animals showed stable metagenomic capacity with maintained transcriptional activity. This discordance suggests that TOL animals achieve metabolic homeostasis through microbial community restructuring toward functional redundancy, rather than energetically costly sustained transcriptional investment. Such a strategy may confer adaptive advantages during the combined metabolic demands of lactation and HS, as transcription and translation are energetically expensive processes.

Metabolomic findings. Sphingolipids exhibited the strongest temporal response across all metabolite classes ($p = 3.6\times10^{-10}$), with sphingomyelins showing a significant genetic line × timepoint interaction ($p = 0.036$). No metabolite guilds showed significant main effects of genetic line, reinforcing that genetic differences manifest primarily through differential temporal response trajectories rather than constitutive metabolic differences. Sphingolipids serve dual roles as structural membrane components and bioactive signaling molecules during cellular stress

responses, suggesting that thermotolerant animals may manage membrane fluidity and cellular signaling differently under thermal challenge.

Implications for genetic selection. Minimal baseline differences between TOL and SEN lines suggest thermotolerance is primarily a differential response capacity rather than a constitutive phenotypic difference. Selection may therefore be more effective under thermal challenge than thermoneutral conditions, where genetic differences emerge more clearly—consistent with heat tolerance being polygenic and with the coordinated guild-level changes we observe.
The capacity–activity discordance—TOL animals expanding functional capacity by recruiting microbial diversity while reducing transcriptional investment—underscores the value of integrated multi-omics. 16S sequencing could capture the compositional shifts but not the transcriptional dynamics; establishing that TOL animals gain function through community expansion rather than greater per-organism activity requires paired metagenomic and metatranscriptomic data. Guild-based frameworks organized by function rather than taxonomy may add resolution for genetic studies by enabling direct comparison of functional potential versus realized activity across backgrounds.

Conclusions

This integrated multi-omics investigation suggests that thermotolerance in lactating sows may represent differential capacity for adaptive microbial community restructuring rather than constitutive metabolic reprogramming. Thermotolerant animals appear to achieve metabolic homeostasis by increasing functional redundancy at the community level while minimizing energetically costly transcriptional investment. Enhanced hindgut methanogenesis, increased fermentation capacity, and efficient stress management emerged as features associated with thermotolerant phenotypes. These findings advance understanding of host-microbiome interactions during environmental stress and provide candidate mechanisms for developing interventions to enhance lactational performance under thermal challenge. Validation in larger cohorts is needed to confirm these preliminary observations.

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