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# Bacterial viability and metabolic profiles of *Lacticaseibacillus casei* AP and *Pediococcus acidilactici* BE under various thermal treatment conditions

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In this study, the effects of heat stress on the viability and metabolic profiles of two indigenous probiotic strains, *Lacticaseibacillus casei* AP and *Pediococcus acidilactici* BE, were assessed using liquid chromatography–high-resolution mass spectrometry. Both strains were subjected to thermal treatment at 37, 55, and 67 °C, followed by viability assessment and untargeted metabolomics analysis. The results indicated that compared to *P. acidilactici* BE, *Lacticaseibacillus casei* AP exhibited superior tolerance and maintained greater viability under heat stress. At 55 °C and 67 °C, *Lacticaseibacillus casei* AP resulted in lower reductions in cell viability, which was supported by adaptive responses involving heat shock proteins and membrane lipid modification. Heatmap visualization, principal component analysis, and partial least squares discriminant analysis revealed distinct metabolite signatures across all strains and temperature conditions. In *P. acidilactici* BE, metabolites such as citric acid, tri (2-ethylhexyl) ester, N-(3-aminopropyl) hexadecanamide, and valine were prominent under stress, whereas *Lacticaseibacillus casei* AP exhibited increased production of nucleotides (e.g., guanosine-5'-monophosphate), peptides (e.g., rhabdopeptide-1), and membrane stabilizing compounds (e.g., monogalactosyl diacylglycerols and sterols). These findings confirm the occurrence of strain-specific metabolic adaptations, with *Lacticaseibacillus casei* AP demonstrating a clear protective mechanism against heat stress. This study provides critical insights into probiotic resilience and offers guidance for the development of stable probiotic formulations capable of withstanding industrial processing and storage at elevated temperatures.

## KEYWORDS

heat stress, *Lacticaseibacillus casei* AP, metabolic profile, microbial viability, *Pediococcus acidilactici* BE, probiotics

## 1 Introduction

Increased access to high-calorie and fast foods has significantly contributed to higher daily caloric intake and a growing prevalence of metabolic syndrome. According to Bruce and Hanson (2010), metabolic syndrome comprises a constellation of symptoms arising from various cardiometabolic risk factors, including obesity, insulin resistance, dyslipidemia, and hypertension. Epidemiological data show that metabolic syndrome affects 20%–25% of the global population. The Framingham Offspring Study reported a prevalence of 29.4% in men and 23.1% in women aged 26–82 years (Ingelsson et al., 2007). In Indonesia, 23.34% of the population suffers from metabolic syndrome, with a prevalence of 26.2% among men and 21.4% among women (Hadaegh et al., 2013). An important consequence of metabolic syndrome is impairment of the immune system, which renders individuals more vulnerable to disease.

Recent advancements in food production extend beyond merely satisfying nutritional requirements; they also prioritize the health benefits of food for humans, enabling these foods to function as functional foods. Functional foods are foods that have been fortified or enriched to improve their nutritional content, thereby fulfilling the nutritional requirements of the body and conferring positive health effects (Hasler, 2002). Examples of functional foods that offer health benefits include those that contain probiotics.

Probiotics are live microorganisms that are incorporated into food products, either individually or in combination, to enhance digestive health. Probiotics are acknowledged for their health benefits to the host when they are administered in adequate amounts ( $10^6$ – $10^7$  CFU/mL) (Food and Agriculture Organization of the United Nations and World Health Organization, 2006). The global probiotic market expanded from USD 79.6 billion in 2024 to USD 86.8 billion in 2025, with projections reaching USD 132.8 billion by 2029 (The Business Research Company, 2024). In Indonesia, the probiotic market is predominantly supplied by imported products, primarily from Europe, Japan, and the United States. This reliance not only elevates retail prices for consumers, but it also poses challenges related to the availability of probiotics and their adaptability to local human gastrointestinal conditions. In contrast, Indonesia possesses a rich microbial biodiversity derived from traditional fermented food products and other local sources. This presents substantial opportunities to develop indigenous probiotics that are not only scientifically competitive, but that also offer adaptive benefits for local consumers.

Notable local probiotics that have been developed to date include *Lactocaseibacillus casei* AP and *Pediococcus acidilactici* BE (Widodo and Taufiq, 2017; Widodo et al., 2019; Widodo et al., 2021; Widodo et al., 2023). *Lactocaseibacillus* is a newly established genus that originated from the reclassification of *Lactobacillus* (Zheng et al., 2020). *Lactocaseibacillus casei* AP was isolated and identified from the feces of naturally born, breastfed Indonesian infants aged less than 1 month. This strain was able to acidify milk resulted in a high viscosity (Widodo Tono et al., 2012; Widodo and Taufiq, 2017). Milk fermented with *Lactocaseibacillus casei* AP has also been reported to be an effective antihypercholesterolemic and antihyperglycemic agent (Widodo et al., 2021). Widodo Septiana et al. (2012) successfully isolated the *P. acidilactici* strain BE from the same feces samples. Milk fermented with *P. acidilactici* BE has been

shown to reduce blood glucose levels in diabetic rats, consistent with increased insulin production and an increase in both the number and percentage of immunoreactive pancreatic beta cells (Widodo et al., 2023).

Probiotics are typically incorporated into food products such as fermented dairy products, which require storage under constant refrigeration along with the implementation of cold chain technology during distribution to consumers. Consequently, the incorporation of probiotics into liquid products is expensive owing to the high costs associated with refrigeration. In addition, the viability of bacterial cells decreases due to excessive acidification and prolonged storage times (Terpou et al., 2019). Therefore, innovations in the development of probiotic powders are important alternatives to liquid formulations (Ferdousi et al., 2013).

Dehydration represents a preservation technique that reduces the water content and activity ( $A_w$ ). A low  $A_w$  value (i.e.,  $<0.6$ ) is essential to avoid the growth of spoilage microorganisms and to extend the shelf life of food products. For example, spray drying is a dehydration technology that removes moisture from food products and decreases their  $A_w$  values. However, the heat treatment employed during spray-drying can decrease the viability of probiotic cells and adversely affect their metabolic activities (Soukoulis et al., 2014). Spray drying involves various parameters, including the inlet air temperature, outlet air temperature, air flow rate, product feed rate, and atomized droplet size (Santivarangkna et al., 2008). Among these parameters, the outlet air temperature affects the viability of spray-dried probiotic cultures the most (Santivarangkna et al., 2008). Low outlet temperatures have been reported to increase productivity (Felfoul et al., 2022) and maintain bacterial viability during spray drying (Ananta et al., 2005; Desmond et al., 2002). Habtegebriel et al. (2018) reported that spray drying at an outlet temperature range of 60 °C–80 °C caused only limited denaturation of milk. In our previous study, we optimized the spray-drying process by setting the inlet air temperature to 160 °C and conditioning the feed pump to maintain an outlet air temperature of 67 °C (unpublished data). This parameter was identified as the most suitable for scaling up production because of its high productivity, short processing time, and ability to maintain both physicochemical quality and microbial viability. The response of probiotics to heat stress during spray drying with an outlet air temperature at 67 °C is therefore important to investigate. In this study, bacterial viability and metabolic profiles under heat treatment at 67 °C were compared with those under normal growth conditions at 37 °C, as well as with those under heat treatment at 55 °C, which was intended to result in 50% bacterial cell injury according to a previous study (Irie et al., 2014).

The metabolic profiles of probiotics reflect their metabolic activity and physiological status under specific conditions. Variations in these profiles can also serve as significant indicators of how probiotics respond and adapt to heat stress. For example, the levels of metabolites, such as glutamate, mannitol, and ethanol, may increase in response to oxidative stress and disruptions in energy metabolism. Using metabolomic techniques based on gas chromatography–mass spectrometry (GC-MS) and liquid chromatography–mass spectrometry (LC-MS), changes in the concentrations of these metabolites have been precisely detected and compared before and after heat treatment (Zhang et al., 2022; Li et al., 2025). Overall, metabolic profiling can serve as a diagnostic

tool for evaluating cellular adaptation to heat stress and indicating physiological resilience. The resulting findings can be used to select probiotic cultures with enhanced metabolic stability and to design fermentation media or process conditions that are optimal for maintaining cell viability and the bioactive functions of probiotics (Timilsena et al., 2020). However, the resistance of *Lactiseibacillus casei* AP and *P. acidilactici* BE to heat stress and their resulting metabolic adaptations remain unknown. Consequently, the aim of the current study is to evaluate the effects of heat stress on the viability and metabolic profiles of *Lactiseibacillus casei* AP and *P. acidilactici* BE.

## 2 Materials and methods

### 2.1 Bacterial culture and growth conditions

Bacterial cultures were propagated in sterilized de Man Rogosa Sharpe (MRS) broth (Merck, Darmstadt, Germany). The *Lactiseibacillus casei* AP or *P. acidilactici* BE cultures were inoculated into test tubes containing sterile MRS broth using a single loop. The inoculated MRS broth medium was then incubated at 37 °C for 20 h. Upon completion of the incubation period, the culture was further propagated in MRS broth to achieve a logarithmic growth phase and harvested as an inoculum.

### 2.2 Heat treatment of *Lactiseibacillus casei* AP and *Pediococcus acidilactici* BE

Bacterial cultures of *Lactiseibacillus casei* AP and *P. acidilactici* BE, each at a concentration of 1% (v/v), were inoculated into sterile MRS broth medium and subsequently incubated at 37 °C until they reached the logarithmic growth phase, which occurred after ~8 h of incubation. For the heat treatment, bacterial suspensions (25.00 mL per 50 mL tube) were immersed in a circulating water bath (Memmert, IN 30; setpoints 37, 55, and 67 °C; stability ±0.1 °C). The bath temperature was verified at each setpoint using a traceable reference digital thermometer (Monotaro; system accuracy ±0.1 °C). The tubes were fully submerged, and gentle rack agitation was applied to ensure thermal homogeneity. The 5-min hold time was measured from the moment the internal temperature in the dummy tube first reached the target, following the methodology of (Irie et al., 2014; Katsui et al., 1982) with modifications. Each temperature condition included three biological replicates and a calibration record.

### 2.3 Assessment of bacterial viability

Before and after heat treatment, the bacterial cell viability was determined by serially diluting an aliquot (100 µL) of the treated culture with a 0.9% NaCl solution (900 µL) to give dilutions ranging from 10<sup>-1</sup> to 10<sup>-8</sup>. The samples were then plated on sterile MRS agar. Each treatment was performed in triplicate. The total viable cells for each condition were determined by the total plate count on the MRS agar plate after incubation at 37 °C for 48 h. The number of colony-forming units (CFUs) was calculated per milliliter of sample.

### 2.4 Metabolomic sample preparation

For metabolomics analysis, the bacterial cells were harvested by centrifugation at 5,000 × g and 30 °C for 15 min. Subsequently, the bacterial cells were collected, vortexed for 1 min, and sonicated at 30 °C for 5 min to facilitate metabolite extraction. In this study, a comprehensive evaluation of lysis efficiency across different strains, utilizing techniques such as live/dead staining, flow cytometry, or microscopy, was not conducted. Consequently, the potential for a minor influence from strain-specific permeabilization or lysis remains a possibility and should be taken into account when interpreting differences in strain-specific metabolites. Following sonication, the samples were subjected to centrifugation at 7,000 × g and 30 °C for 15 min, and the supernatant was filtered through a 0.20 µm nylon filter. The filtrates were then used for further metabolic profiling.

### 2.5 Metabolic profiling

Untargeted metabolite profiling was performed using a Thermo Scientific™ Vanquish™ Horizon UHPLC system coupled with an Orbitrap™ Exploris 240 High-Resolution Mass Spectrometer (HRMS; Thermo Fisher Scientific, Massachusetts, United States). Chromatographic separation was achieved using a Thermo Scientific™ Accucore™ C18 column (100 mm × 2.1 mm, 2.6 µm particle size; Thermo Fisher Scientific, Massachusetts, USA) maintained at 40 °C. The mobile phases consisted of MS-grade water containing 0.1% formic acid (mobile phase A) and MS-grade acetonitrile containing 0.1% formic acid (mobile phase B), at a flow rate of 0.3 mL/min. The gradient was started at 5% B, was increased to 90% B over 16 min, and was maintained for 4 min before returning to 5% B over 25 min. The injection volume was 5 µL. HRMS was performed in full MS/dd-MS<sup>2</sup> mode with polarity switching, capturing the spectra at a resolution of 60,000 full width at half maximum (FWHM) across an *m/z* range of 70–800. Data-dependent fragmentation (dd-MS<sup>2</sup>) was conducted at 30,000 FWHM using normalized collision energies of 30, 50, and 70. The ion source was set to 3500 V for the positive mode and 2500 V for the negative mode. All other parameters were optimized for metabolite detection.

The MS data were processed using Compound Discoverer 3.3 software (Thermo Fisher Scientific). Features were detected with a 5 ppm mass tolerance and grouped by retention time with a tolerance of 0.2 min. The compounds were annotated using ChemSpider databases, including FooDB and the LipidMap Structure Database. The MS<sup>2</sup> spectra were matched to the mzCloud database for further compound annotation. Chemometric analysis was performed using the web-based software package, Metabo-Analyst 6.0. Data were normalized using the sum method, followed by logarithmic transformation and autoscaling. Partial least squares discriminant analysis (PLS-DA) and principal component analysis (PCA) were used to distinguish samples subjected to different heat stress treatments.

### 2.6 Statistical analysis

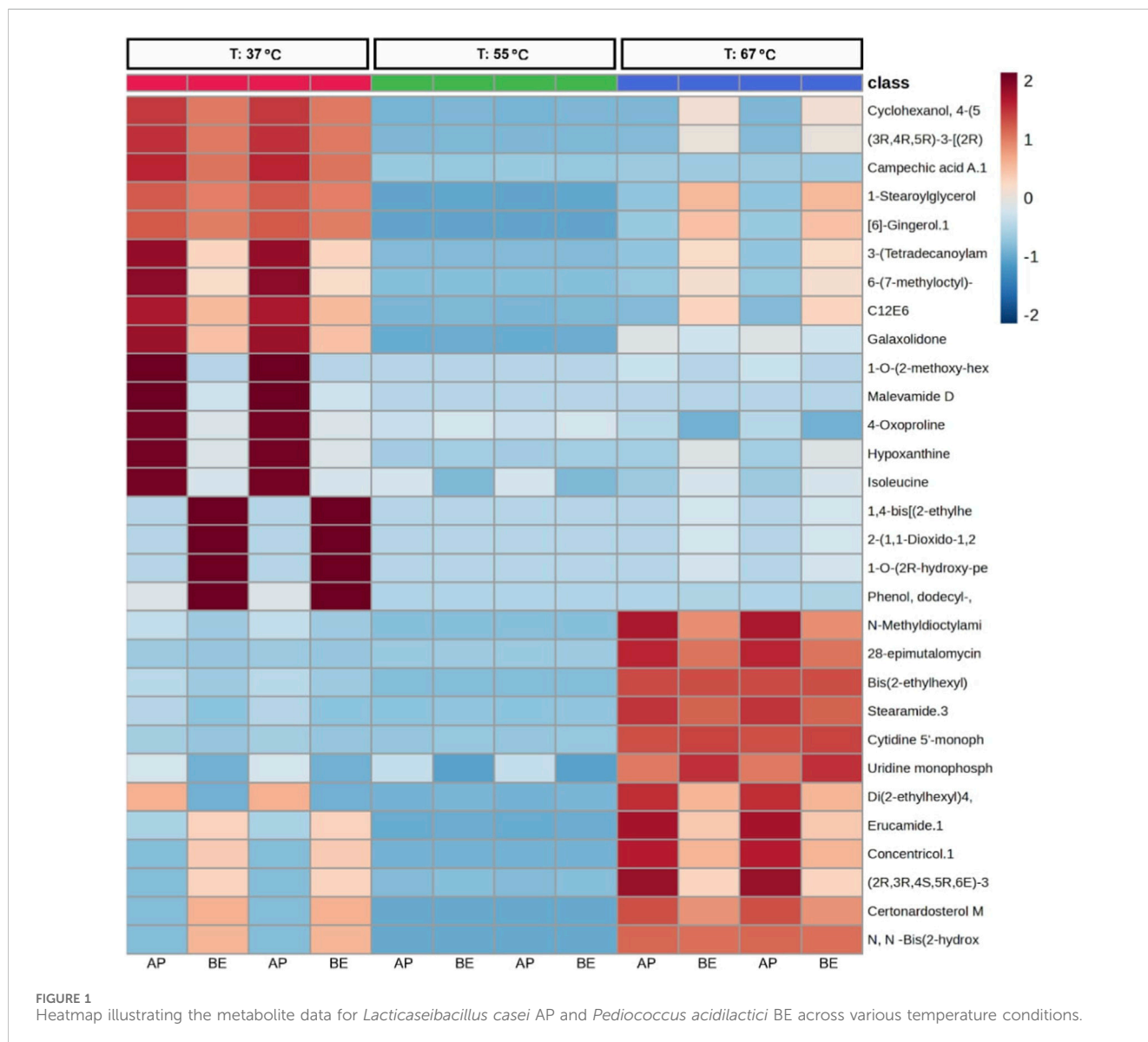
Statistical analysis of probiotic cell viability under different heat stress treatment conditions was performed using one-way analysis of

TABLE 1 Bacterial viability of *Lacticaseibacillus casei* AP and *Pediococcus acidilactici* BE cells following heat treatment.

Probiotic culture	Bacterial viability (log CFU/mL)		
	37 °C <sup>ns</sup>	55 °C	67 °C
<i>L. casei</i> AP	8.51 ± 0.03	8.36 ± 0.03 <sup>b</sup> (Δ -7.5%)	7.96 ± 0.06 <sup>b</sup> (Δ-11.9%)
<i>P. acidilactici</i> BE	8.45 ± 0.03	8.03 ± 0.11 <sup>a</sup> (Δ-11.1%)	7.58 ± 0.12 <sup>a</sup> (Δ-16.1%)

ns, not significant.

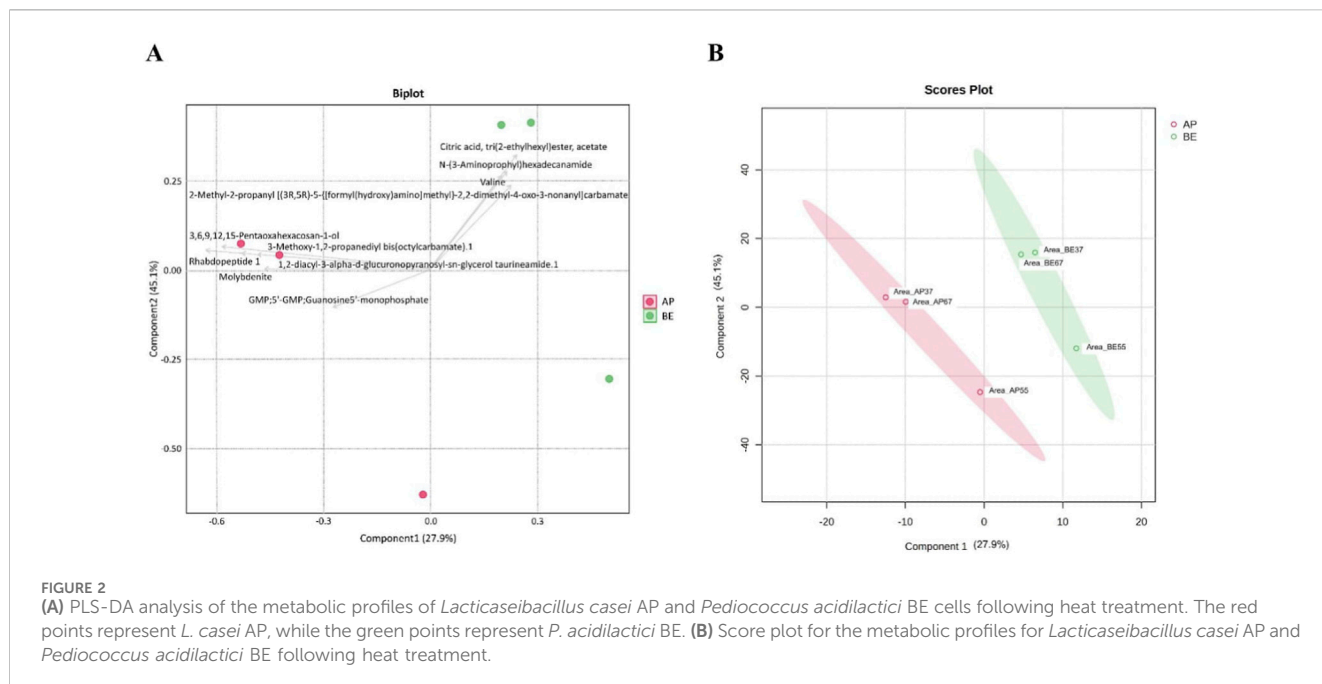
ab: Different superscript letters within the same column denote statistically significant differences (*p* < 0.05).



variance (ANOVA), with the significance level set at  $\alpha = 0.05$ . This analysis was followed by Tukey’s HSD *post hoc* test to facilitate pairwise comparisons. The data, obtained from multiple experimental replicates, are reported as the mean ± standard deviation (SD). A *p* value of <0.05 indicated that heat stress had a discernible effect on the viability of *Lacticaseibacillus casei* AP and *P. acidilactici* BE.

### 3 Results

Heat stress is a critical environmental challenge for bacterial cells during spray drying, since temperatures exceeding 40 °C can disrupt the integrity of cellular macromolecules, including proteins and lipids, within the cell membrane. Table 1 shows the viability of the *Lacticaseibacillus casei* AP and *P. acidilactici* BE cells after heat



treatment under different conditions. As detailed in the table, both *Lactocaseibacillus casei* AP and *P. acidilactici* BE cultures experienced a decrease in cell viability after growth at elevated temperatures. Specifically, *Lactocaseibacillus casei* AP experienced 7.5% and 11.9% decreases at 55 °C and 67 °C, respectively. In contrast, *P. acidilactici* BE experienced 11.1% and 16.1% decreases at the same temperatures. Both cultures exhibited similar bacterial viabilities prior to heat treatment when both strains were incubated at 37 °C (Table 1).

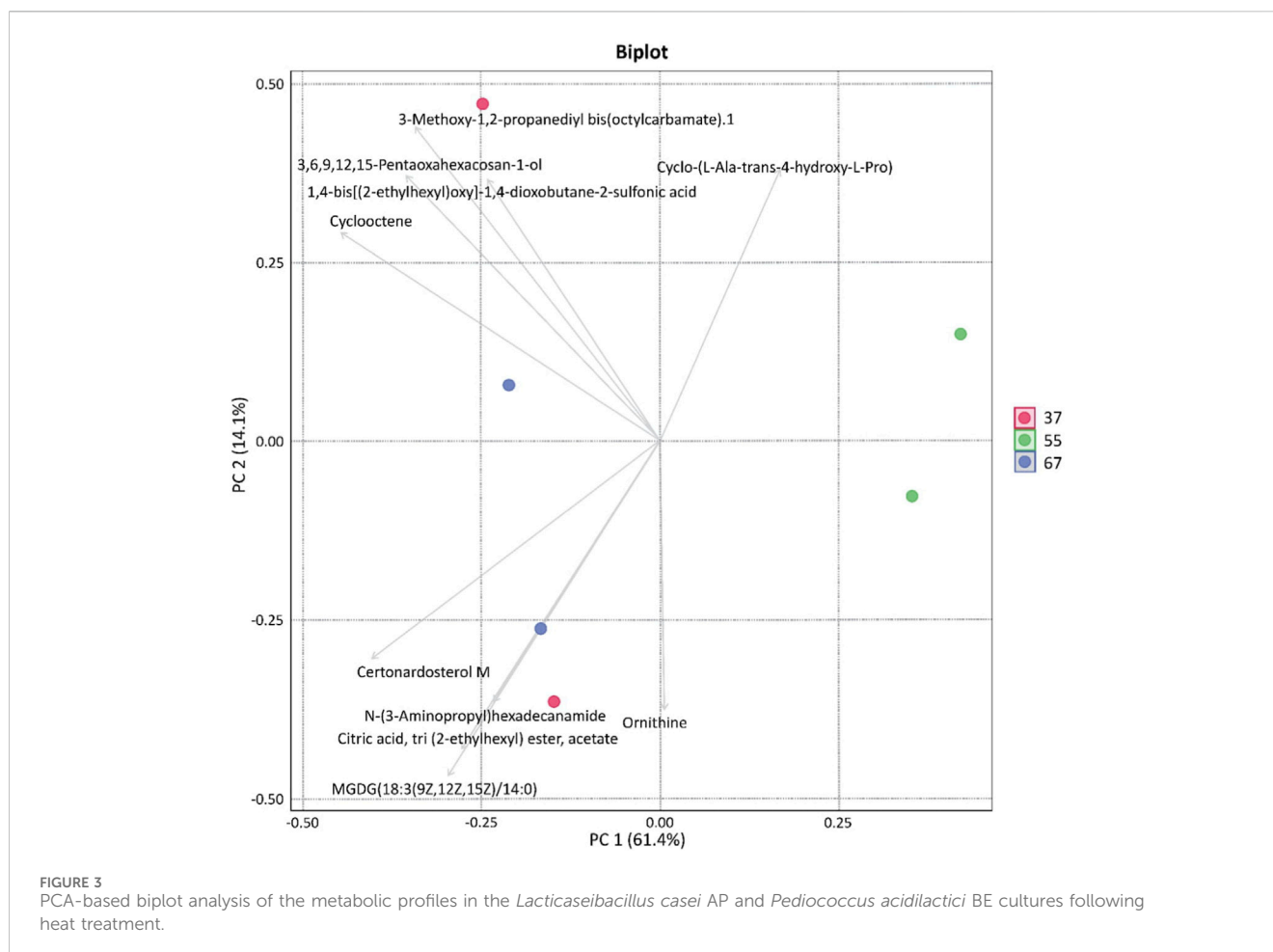
The metabolic profiles of *Lactocaseibacillus casei* AP and *P. acidilactici* BE were analyzed using a heatmap and are presented in Figure 1. Specifically, the metabolic profiles are shown for these cultures after heat treatment at 37, 55, and 67 °C. In this analysis, dark red indicates a high concentration of metabolites, whereas dark blue indicates a low concentration. The metabolic profiles obtained at 37 °C indicate that *Lactocaseibacillus casei* AP and *P. acidilactici* BE produce primary metabolites such as isoleucine, hypoxanthine, 4-oxoproline, cyclohexanol, campechic acid, gingerol, galaxolidone, and maleamide D (Figure 1). These observations suggest that these metabolites are closely associated with essential metabolic processes, particularly amino acid and purine metabolism (Kim et al., 2024; Aoki et al., 2025).

Compared with the profile obtained at 37 °C, the metabolic profile recorded at 55 °C was substantially altered in both strains (Figure 1). Metabolites that were present at high concentrations at 37 °C underwent a marked reduction, with decreases in the concentrations of cyclohexanol, campechic acid, and gingerol being particularly pronounced, as evidenced by the predominance of blue coloration. These reductions suggest that the bacterial cells encountered thermal stress, which resulted in modifications to their metabolic pathways, reduced enzymatic activity, or adjustments in metabolism as an adaptive response to elevated temperature conditions (Castaldo et al., 2006; Liu et al., 2021).

The metabolic profile observed at 67 °C exhibited significant alterations, notably in the levels of nucleotide metabolites such as

cytidine 5'-monophosphate and uridine monophosphate, as well as lipid compounds such as erucamide, certonardosterol, N,N-bis(2-hydroxyethyl), and additional compounds such as 28-epimutalomycin and phenol dodecyl (Figure 1). This increase in nucleotide metabolites is intricately linked with bacterial responses to heat-induced DNA stress, including mechanisms like DNA repair and the synthesis of new RNA as a protective response (Qian et al., 2014).

Multivariate analysis was subsequently performed using PLS-DA to compare the metabolic profiles of *Lactocaseibacillus casei* AP and *P. acidilactici* BE after heat treatment. PLS-DA is a statistical method designed to differentiate sample groups based on their metabolic patterns and identify metabolites that significantly contribute to these distinctions. The outcomes of the PLS-DA analysis are shown in Figure 2, wherein the effects of the different heat treatment temperatures on the metabolic patterns are clearly illustrated. The PLS-DA results can be divided into two components, namely component 1 and component 2. In the principal component analysis (PCA) biplot depicted in Figure 2A, a distinct separation between strains is evident along PC1, which accounts for 27.9% of the variance, with an additional contribution from PC2 at 45.1%. Replicates of *P. acidilactici* BE (represented by green dots) are clustered predominantly on the right side of the plot, mainly within the upper right quadrant, and exhibit a positive correlation with several features that load strongly in the same direction, including citric acid, tri(2-ethylhexyl) ester, N-(3-aminopropyl) hexadecanamide, and valine. In contrast, replicates of *Lactocaseibacillus casei* AP (indicated by red dots) are clustered on the lower left side and are associated with features exhibiting negative loadings on PC1/PC2, such as guanosine-5'-monophosphate (GMP), rhabdopeptide-1, and several other minor components. This pattern suggests that the variation in metabolites projected onto PC1 is the primary driver of discrimination between strains, with *P. acidilactici* BE demonstrating relatively high levels of certain amino/amide



compounds and esters, whereas *Lactocaseibacillus casei* AP is more closely associated with specific nucleotides and peptides. The broader distribution of *P. acidilactici* BE points along the PC2 axis indicates slightly greater metabolic heterogeneity in this strain than in *Lactocaseibacillus casei*, yet both groups remain consistently separated, which confirms the presence of distinct metabolic signatures for each strain.

Additionally, PLS-DA clearly revealed differences in the metabolic profiles at the three treatment temperatures. As shown in Figure 2B, area\_AP37 and area\_AP67 are closely positioned in the upper-left quadrant of the plot, whereas area\_AP55 is notably displaced downward, albeit still within the AP-class ellipse. The distinct positioning of area\_AP55 suggests that at 55 °C, the *Lactocaseibacillus casei* AP strain experiences a marked alteration in metabolite expression, differing from the profiles observed at 37 °C and 67 °C. This implies that a temperature of 55 °C represents a critical physiological threshold, potentially triggering stress responses, such as the synthesis of protective metabolites or metabolic adjustments. The metabolic profiles of *P. acidilactici* BE cultures were tightly clustered in the upper right quadrant of the plot, indicating clear homogeneity, although area\_BE55 was positioned slightly lower and remained within the same cluster, indicating resilience to heat stress. Compared with the *Lactocaseibacillus casei* AP cultures, the *P. acidilactici* BE cultures exhibit greater metabolic stability across all temperatures, including

at 55 °C. These results imply that *P. acidilactici* BE potentially sustains a consistent core metabolic function when it is subjected to heat stress. Nevertheless, distinct protective strategies or alterations in metabolite biosynthesis under these conditions seem to be lacking.

Multivariate analysis was performed to evaluate the metabolic profiles after subjecting the cultures to various heat-stress temperatures. The results were evaluated using PCA, as presented in Figure 3. Notably, PCA reduces the dimensionality of a large dataset into several principal components (PCs) that encapsulate the most significant variations within the data. The first principal component (PC1) and second principal component (PC2) explained 61.4% and 14.1% of the total data variance, respectively. The colored points on the plot correspond to the different temperature treatments, wherein red represents 37 °C, green represents 55 °C, and blue represents 67 °C. The observed shifts in the positions of these points within the plot indicates notable differences in the metabolic profiles across the temperature spectrum, wherein greater separation between points represents more pronounced differences. PCA modeling indicated separation driven by temperature on PC1 and strain-specific modulation on PC2 (variance proportions are displayed on the axes of Figure 3). In essence, the score plot is depicted in Figure 4, while Figure 3 clarifies the link between score separation and the direction and magnitude of metabolite contributions (loading), with

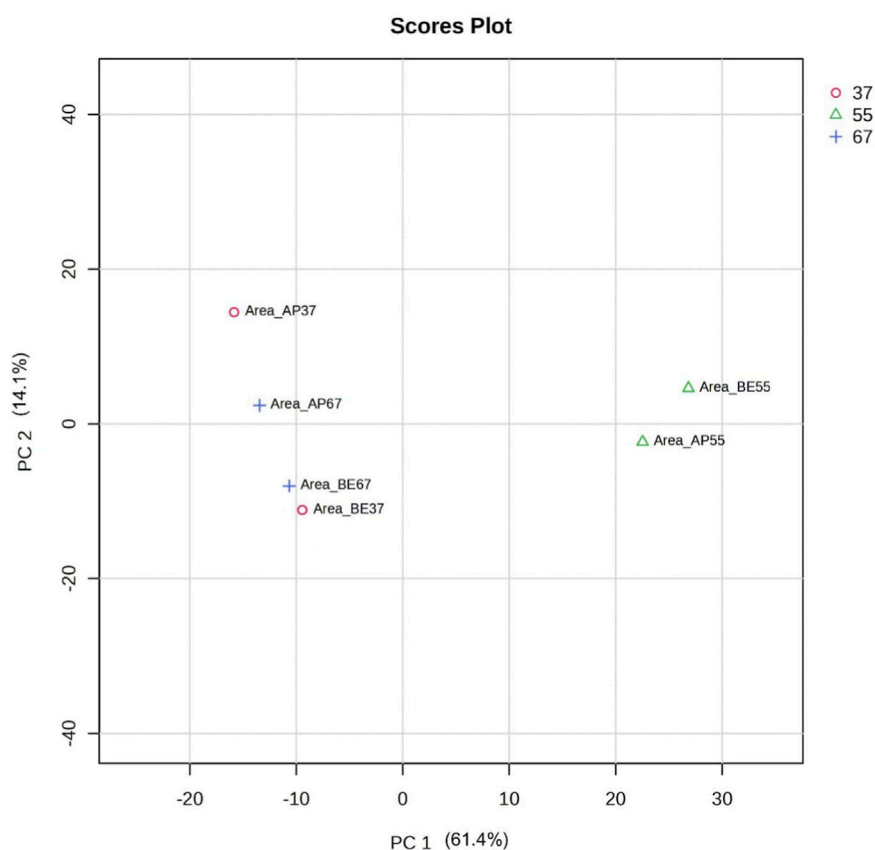


FIGURE 4 Illustration of the metabolic profile scores in cultures of *Lactocaseibacillus casei* AP and *Pediococcus acidilactici* BE exposed to different heat stress conditions.

the 95% confidence ellipses for each group assisting in visualizing the consistency of replicates and the proximity of clusters.

Figure 4 provides a visual representation of the sample distribution based on the scores or projection values for PC1 (61.4%) and PC2 (14.1%). Unlike a biplot that includes metabolite vectors, a score plot focuses solely on the positioning of each sample, thereby accentuating separation and grouping according to the treatment conditions. Each point on the plot corresponds to an individual sample; area\_AP37B corresponds to *Lactocaseibacillus casei* AP at 37 °C, area\_BE37B denotes *P. acidilactici* BE at 37 °C, area\_AP55B represents *Lactocaseibacillus casei* AP at 55 °C, area\_BE55B indicates *P. acidilactici* BE at 55 °C, area\_AP67B refers to *Lactocaseibacillus casei* AP at 67 °C, and area\_BE67B pertains to *P. acidilactici* BE at 67 °C. Primary separation is observed along PC1 (61.4%), which is predominantly influenced by temperature. Both strains exhibit a marked shift to the right (positive PC1) at 55 °C, with *Lactocaseibacillus casei* AP and *P. acidilactici* BE demonstrating the most significant alterations in metabolite profiles compared with the conditions at 37 °C and 67 °C. In contrast, the conditions at 37 °C and 67 °C tend to cluster on the left (negative PC1), with *Lactocaseibacillus casei* AP at both temperatures grouped in the left quadrant and *P. acidilactici* BE at 37 °C and 67 °C positioned within the lower PC1 range. The secondary separation along PC2 (14.1%) highlights differences in the strain responses at specific temperatures. At 37 °C, the abundance of *Lactocaseibacillus*

*casei* AP is greater (positive PC2) than that of *P. acidilactici* BE (negative PC2), indicating that distinct metabolite components differentiate the two strains under basal conditions. Conversely, at 55 °C, compared with *Lactocaseibacillus casei* AP, *P. acidilactici* BE showed a slightly higher PC2 score, suggesting a strain–temperature interaction in the metabolite response patterns. At 67 °C, both strains are positioned near the PC2 axis (approximately zero to slightly negative), indicating reduced interstrain variation compared with that at 37 °C and 55 °C. Overall, this pattern confirms that temperature, particularly 55 °C, is the primary driver of metabolomic variation (PC1), while strain-specific differences (PC2) modulate responses at certain temperatures. These findings align with the results of the present study, which revealed distinct metabolite signatures for each strain under heat stress.

## 4 Discussion

*Lactocaseibacillus casei* AP and *P. acidilactici* BE exhibited similar bacterial viabilities prior to heat treatment when both strains were incubated at 37 °C (Table 1). This observation confirmed the equivalent growth rates of both cultures under standard temperature conditions, ensuring that any subsequent variations could be clearly attributed to heat treatment (Tripathi and Giri, 2014). At 55 °C, the *Lactocaseibacillus casei* AP cells

exhibited significantly greater viability than the *P. acidilactici* BE cells. Increasing the temperature to 67 °C revealed a clearer difference in viability between the two strains, with the *P. acidilactici* BE culture experiencing a substantial growth decline relative to *Lactocaseibacillus casei* AP. These findings suggest that *Lactocaseibacillus casei* AP cells are more tolerant than *P. acidilactici* BE cells when exposed to temperatures of 55 °C and 67 °C. The differences in heat stress tolerance among the bacterial cultures can be partially attributed to the proportion of saturated fatty acids present in the cell membrane. Previous studies by Adu et al. (2018) and Zhang et al. (2021) indicated that *Lactocaseibacillus casei* cultures possess a relatively high concentration of saturated fatty acids, which contributes to membrane stability at moderate temperatures. In contrast, *P. acidilactici* requires external protection to preserve its membrane fluidity (Adu et al., 2018; Zhang et al., 2021).

Heatmap analysis represents a comprehensive method for assessing variations in metabolic expression patterns across different samples or treatments. Specifically, a heatmap visually represents the relative intensities of various metabolites identified in research samples, with red indicating a high level of metabolite expression and blue indicating a low level of expression (Goodacre et al., 2004). Such analysis offers a clear visual depiction of sample clustering based on similarities in the metabolic profiles, thereby facilitating the interpretation of the physiological changes resulting from heat treatment (Liu et al., 2025). Comparative analysis of the metabolic profiles of the *Lactocaseibacillus casei* AP and *P. acidilactici* BE strains revealed distinct metabolic responses to heat stress in each strain (Figure 1). The heatmap shows that compared with *P. acidilactici* BE, *Lactocaseibacillus casei* AP exhibits a more robust response, particularly at 67 °C. This enhanced adaptation in the cells of *Lactocaseibacillus casei* AP was evidenced by significant alterations in the metabolite intensity, including those related to nucleotides and membrane lipids, whereas *P. acidilactici* BE demonstrated comparatively moderate changes in the metabolite intensity. According to a previous study by Adu et al. (2018), *Lactocaseibacillus casei* possesses an effective protective mechanism against heat stress, such as the elevated expression of heat shock proteins. In contrast, *P. acidilactici* is less heat tolerant (Jonathan et al., 2023).

Heatmap analysis of the metabolic profiles revealed that exposure to high-temperature stress induced substantial alterations in the metabolic profiles of both probiotic strains. The observed responses included increases in the levels of nucleotide metabolites, membrane lipids, and other protective compounds that function as mechanisms for cellular adaptation. Nucleotides such as cytidine 5'-monophosphate and uridine monophosphate generally serve to safeguard bacterial cells and facilitate adaptation to heat stress by regulating secondary metabolism and synthesizing the nucleotides necessary for the repair and protection of critical molecular structures within the cell (Zhen et al., 2020). Moreover, an increased lipid intensity (e.g., for erucamide and cerotanordersterol) suggests that the bacteria actively modify their cell membrane structure to maintain membrane fluidity, which is crucial for preserving cellular integrity under high-temperature conditions (Fonseca et al., 2019; Siroli et al., 2020). Consequently, augmentation of these metabolites is part of the intricate adaptive mechanisms employed by bacteria in response to severe heat stress.

Compared with *P. acidilactici* BE, *Lactocaseibacillus casei* AP demonstrated a more pronounced metabolic response, suggesting significant differences in the metabolic strategies and adaptation mechanisms employed by each strain. As reported by Liao et al. (2010), heat stress compels probiotic bacteria to modify their metabolism by enhancing the synthesis of secondary metabolites, such as nucleotides and lipid compounds, which ultimately preserves membrane integrity and protects the cellular structure against heat stress-induced damage.

Using PLS-DA analysis, different dominant metabolites were observed between the two probiotic strains. Specifically, citric acid, tri (2-ethylhexyl)ester, N-(3-aminopropyl)hexadecanamide, and valine were detected as metabolites in the *P. acidilactici* BE culture, whereas pentaohexacosan-1-ol, rhabdopeptide 1, guanosine-5'-monophosphate (5'-GMP), and molybdenite were detected in the *P. acidilactici* BE culture. Citric acid and valine represent critical metabolites in the tricarboxylic acid cycle and protein biosynthesis pathways, suggesting that *P. acidilactici* BE produces more energy through primary metabolism in response to osmotic stress conditions under increased growth temperatures (Cronan, and Laporte, 2005; Ye et al., 2012; Akram, 2014). Moreover, 5'-GMP and peptides (e.g., rhabdopeptides), which are linked to metabolic regulation and antimicrobial activity, are likely involved in the adaptive responses of *Lactocaseibacillus casei* AP to elevated temperatures (Rossi et al., 2016). It is also known that monogalactosyl diacylglycerols, including galactolipid derivatives such as 1,2-diacyl-3- $\alpha$ -glucopyranosyl-sn-glycerol, play a significant role in preserving membrane integrity during heat stress. Additionally, elevated levels of sterol compounds in *Lactocaseibacillus casei* have been reported to fortify membranes and mitigate ion loss at extreme temperatures (Papadimitriou et al., 2016). Sterol compounds increase membrane stability and protect lipids from maintaining cellular homeostasis. Moreover, in *Lactobacillus casei*, the ratio of saturated to unsaturated fatty acids increases at extreme temperatures, which helps maintain membrane fluidity and stability (Machado et al., 2004). The metabolism of citric acid in lactic acid bacteria is altered under stress conditions. Citric acid plays a role in mitigating oxidative damage commonly associated with heat stress and contributes to the stabilization of proteins at elevated temperatures (Książek, 2024). N-(3-aminopropyl) hexadecanamide, a fatty acid derivative, is recognized for its ability to modulate membrane properties in response to stress. Long-chain amides can physically influence the membrane, thereby reducing permeability at high temperatures (Bandi et al., 2025). Rhabdopeptide-1 functions as a protease inhibitor, limiting excessive proteolysis and modulating the microbial community when heat stress induces protein lysis (Reimer et al., 2013). MGDG undergoes dynamic changes under heat stress through lipid remodeling mechanisms to preserve membrane fluidity and integrity as temperatures increase. Lactic acid bacteria adapt their membrane lipids in response to heat stress (Walczak-Skierska et al., 2020). Nucleotides, such as 5'-GMP, serve as precursors for (p)ppGpp alarmones involved in the stringent response. The levels of (p)ppGpp increase rapidly during heat shock, aiding bacteria in adapting to the proteotoxicity induced by heat stress (Schäfer et al., 2020). Valine acts as a precursor for branched-chain fatty acids (BCFAs), and an increased proportion of BCFAs stabilizes the membrane and reduces permeability at high

temperatures, a process known as homeoviscous adaptation, thereby increasing heat tolerance. Alterations in membrane fatty acid composition represent a response of lactic acid bacteria to heat stress (Walczak-Skierska et al., 2020).

A previous study reported that *Lactocaseibacillus casei* employs multiple strategies to counteract heat stress, such as increasing the expression of heat shock proteins, modifying membrane lipids, and activating purine metabolic pathways (Desmond et al., 2004; Adu et al., 2018). Modifications in the membrane lipid composition contribute to the maintenance of membrane stability at elevated temperatures. Specifically, heat stress induces changes in the lipid composition, particularly in the synthesis of fatty acids, which promote membrane stability and protect against thermal damage. Moreover, Jonathan et al. (2023) reported that *P. acidilactici* maintains substantial cell viability under heat stress conditions up to 60 °C, with marked reductions being observed at 75 °C and 90 °C. However, the expression of heat shock protein genes, such as groEL, did not change significantly under heat stress conditions.

The vectors depicted by arrows in the PCA-based biplot shown in Figure 3 illustrate the direction and magnitude of the influence of each specific metabolite on the bacterial metabolic profile. The length of each vector corresponds to the strength of the contribution of the specific metabolite (PC), with longer vectors indicating a more substantial contribution. The predominant metabolites in the negative PC1 included certonardosterol M, N-(3-aminopropyl)hexadecanamide, citric acid tri(2-ethylhexyl) ester acetate, and monogalactosyl diacylglycerol (MGDG). These metabolites were more frequently observed at temperatures corresponding to the negative side of PC1. Conversely, the dominant metabolites in the positive PC2 included methoxy-2-propanediyl bis(octylcarbamate).1, cyclo-(L-ala-trans-4-hydroxy-L-pro), cyclooctene, 1,4-bis[(2-ethylhexyl)oxy]-1,4-dioxobutane-2-sulfonic acid, and 3,6,9,12,15-pentaoxahexacosan-1-ol. These findings suggest that the above compounds play a significant role in the bacterial response to specific temperatures and that their levels tend to increase at increasing temperatures. The metabolite ornithine, which aligns with the negative PC2, also significantly contributes to the variation in the data and is associated with metabolic responses to elevated temperatures. The findings of this analysis revealed substantial differences in the metabolic profiles produced by bacteria at 37, 55, and 67 °C. Specifically, each temperature elicited a distinct set of metabolites in response to heat stress adaptation, indicating that heat stress induced specific alterations in their metabolic pathways, as evidenced by the changes in the metabolic profiles. As reported previously, the metabolic profiles of various probiotics are significantly affected by temperature (Papadimitriou et al., 2016). For example, MGDG serves as a crucial membrane modulator that facilitates the maintenance of lipid fluidity at elevated temperatures, consistent with the adaptation mechanisms observed in thermotolerant organisms (Sun et al., 2022).

Figure 4 shows a PCA-based score plot that captures the changes in metabolite levels across a range of temperatures. The samples subjected to heat treatment at 55 °C (area\_AP55B and area\_BE55B) formed a distinct cluster in the upper right quadrant of Figure 4. These observations suggest that compared with exposure to temperatures of 37 °C and 67 °C, exposure at 55 °C elicits markedly different metabolite responses in both *Lactocaseibacillus*

*casei* AP and *P. acidilactici* BE. This temperature can therefore be classified as the active metabolite stress zone. Conversely, the samples exposed to a temperature of 67 °C (area\_AP67B and area\_BE67B) were positioned in the lower left quadrant, indicating a unique metabolite pattern associated with high heat stress, distinct from that observed at 37 °C. This finding implies the existence of an emergency metabolic response, potentially involving increases in the levels of protective lipids or adaptive molecules, such as MGDG, sterols, and lipid amines. At 37 °C, the samples (area\_AP37B and area\_BE37B) were somewhat separated but remained within the basal or non-stress metabolite zone, characterized by a metabolic profile that was indicative of basic cellular metabolism. The results of this score plot analysis reveal that metabolite responses vary with temperature and culture conditions; heat stress at 55 °C can be considered a transitional temperature that induces the active expression of protective metabolites, whereas heat stress at 67 °C activates specific metabolic pathways distinct from those at 37 °C and 55 °C, leading to the activation of protective metabolites.

Under heat stress conditions, there is a notable increase in the levels of ether compounds, which serve as protectors of cell membranes by reducing the surface tension between the lipid molecules within the membrane (Gianni de Carvalho et al., 2019; Yang et al., 2021). Similarly, the levels of sterol compounds increased significantly at elevated temperatures, suggesting that enhanced activation of the sterol biosynthesis pathway plays a role in fortifying the membrane structure (Lee et al., 2018; Abedin et al., 2023). Additionally, the secondary metabolites eucaramide and campechic acid contribute to oxidative homeostasis and function as antioxidants, thereby providing essential biochemical protective responses that enable cell survival under high-temperature conditions (Ou et al., 2012; Fadlillah et al., 2021). Collectively, the interplay between lipid metabolites, sterols, and heat shock protein expression can serve as a metabolic marker to assess the quality and resilience of probiotics during high-temperature processing or in response to heat stress.

In conclusion, this study advances our understanding of how two probiotic strains, namely *Lactocaseibacillus casei* AP and *P. acidilactici* BE, respond metabolically to heat stress. This work highlights not only the distinct adaptive capabilities of these strains at relatively high temperatures, but also the essential compounds that play a role in cellular defense mechanisms. The insights gained from this investigation lay crucial groundwork for developing probiotic applications that can withstand extreme environmental conditions. Several key conclusions could be drawn from the obtained results. Specifically, compared with *P. acidilactici* BE, *Lactocaseibacillus casei* AP demonstrates superior viability at elevated temperatures (i.e., 55 °C and 67 °C), implying that a more robust thermal stress protection mechanism exists in *Lactocaseibacillus casei* AP. Compounds such as citric acid, tri(2-ethylhexyl) ester, N-(3-aminopropyl) hexadecanamide, and valine in *P. acidilactici* BE, as well as 5'-GMP, rhabdopeptide1, and 1,2-diacyl-3- $\alpha$ -glucopyranosyl-sn-glycerol (a monogalactosyl diacylglycerol derivative) in *Lactocaseibacillus casei* AP, may act as metabolic markers that are indicative of responses to increased temperatures. Moreover, the presence of sterol compounds under heat stress conditions suggests that thermal stress triggers changes in the lipid composition of the cell membrane as a primary adaptive

response in *P. acidilactici* BE cells, whereas the synthesis of heat shock proteins in *Lacticaseibacillus casei* AP supports cellular functions during stress. In the industrial application of heat marker metabolites, compounds such as 5'-GMP, rhabdopeptide-1, and MGDG derivatives for *Lacticaseibacillus casei* AP, as well as valine and N-(3-aminopropyl) hexadecanamide for *P. acidilactici* BE, serve as distinctive markers for the efficient identification of robust strains via LC-HRMS. Additionally, the systematic observation of these metabolites during production enables the fine-tuning of fermentation and drying parameters, including the regulation of inlet and outlet temperatures, air flow rates, and the proportion of protectants from proteins or polysaccharides, with the objective of optimizing the viability and stability of the end product.

## Data availability statement

The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding author.

## Author contributions

SR: Methodology, Data curation, Visualization, Investigation, Formal Analysis, Writing – original draft, Project administration, Conceptualization, Writing – review and editing. SW: Conceptualization, Writing – review and editing, Supervision, Funding acquisition, Methodology, Formal Analysis, Writing – original draft, Resources. WW: Methodology, Supervision, Conceptualization, Writing – review and editing, Formal Analysis, Resources, Writing – original draft, Funding acquisition.

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## Conflict of interest

The author(s) declared that this work was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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## References

- Abedin, M. M., Chourasia, R., Phukon, L. C., Sarkar, P., Ray, R. C., Singh, S. P., et al. (2023). Lactic acid bacteria in the functional food industry: biotechnological properties and potential applications. *Crit. Rev. Food Sci. Nutr.* 64, 10730–10748. doi:10.1080/10408398.2023.2227896
- Adu, K. T., Wilson, R., Nichols, D. S., Baker, A. L., Bowman, J. P., and Britz, M. L. (2018). Proteomic analysis of *Lactobacillus casei* GCRL163 cell-free extracts reveals a SecB homolog and other biomarkers of prolonged heat stress. *PLoS One* 13, e0206317. doi:10.1371/journal.pone.0206317
- Akram, M. (2014). Citric acid cycle and role of its intermediates in metabolism. *Cell Biochem. Biophys.* 68, 475–478. doi:10.1007/s12013-013-9750-1
- Ananta, E., Volkert, M., and Knorr, D. (2005). Cellular injuries and storage stability of spray-dried *Lactobacillus rhamnosus* GG. *Int. Dairy J.* 15 (4), 399–409. doi:10.1016/j.idairyj.2004.08.004
- Aoki, K., Mutaguchi, Y., Hemmi, H., Yoshimura, T., and Ito, T. (2025). Identification and characterization of a novel d-Branched-Chain amino acids importer from *Lactobacillus fermentum*. *ChemBioChem* 26, e202401075. doi:10.1002/cbic.202401075
- Bandi, S., Schlemper-Scheidt, M. D., Rivera Sánchez, R., Sutour, S., Glauser, G., Ishida, Y., et al. (2025). Glycosylated N-Acyl phosphoethanolamines as bacterial food-dependent signaling molecules in caenorhabditis nematodes. *ACS Bio Med Chem Au* 5, 602–619. doi:10.1021/acsbiochemau.5c00012
- Bruce, K. D., and Hanson, M. A. (2010). The developmental origins, mechanisms, and implications of metabolic syndrome. *J. Nutr.* 140, 648–652. doi:10.3945/jn.109.111179
- Castaldo, C., Siciliano, R. A., Muscariello, L., Marasco, R., and Sacco, M. (2006). CcpA affects expression of the groESL and dnaK operons in *Lactobacillus plantarum*. *Microb. Cell Fact.* 5, 35. doi:10.1186/1475-2859-5-35
- Cronan, J. E., and Laporte, D. (2005). Tricarboxylic acid cycle and glyoxylate bypass. *EcoSal Plus* 1, 10.1128/ecosalplus.3.5.2. doi:10.1128/ecosalplus.3.5.2
- Desmond, C., Ross, R. P., O'Callaghan, E., Fitzgerald, G., and Stanton, C. (2002). Improved survival of *Lactobacillus paracasei* NFBC 338 in spray-dried powders containing gum acacia. *J. Appl. Microbiol.* 93 (6), 1003–1011. doi:10.1046/j.1365-2672.2002.01782.x
- Desmond, C., Fitzgerald, G. F., Stanton, C., and Ross, R. P. (2004). Improved stress tolerance of GroESL-overproducing *Lactococcus lactis* and probiotic *Lactobacillus paracasei* NFBC 338. *Appl. Environ. Microbiol.* 70, 5929–5936. doi:10.1128/AEM.70.10.5929-5936.2004

- Fadlillah, H. N., Nuraida, L., Sitanggang, A. B., and Palupi, N. S. (2021). Production of antioxidants through lactic acid fermentation: current developments and outlook. *Ann. Univ. Dunarea de Jos Galati, Fascicle VI Food Technol.* 45, 203–228. doi:10.35219/foodtechnology.2021.2.13
- Felfoul, I., Burgain, J., Perroud, C., Gaiani, C., Scher, J., Attia, H., et al. (2022). Impact of spray-drying conditions on physicochemical properties and rehydration ability of skim dromedary and cow's milk powders. *Dry. Technol.* 40 (3), 665–677. doi:10.1080/07373937.2020.1828448
- Ferdousi, R., Rouhi, M., Mohammadi, R., Mohamad Mortazavian, A., Khosravi-Darani, K., and Homayouni Rad, A. (2013). Evaluation of probiotic survivability in yogurt exposed to cold chain interruption. *Iran. J. Pharm. Res.* 12, 139–144.
- Fonseca, F., Pénicaud, C., Tymczyszyn, E. E., Gómez-Zavaglia, A., and Passot, S. (2019). Factors influencing the membrane fluidity and the impact on production of lactic acid bacteria starters. *Appl. Microbiol. Biotechnol.* 103, 6867–6883. doi:10.1007/s00253-019-10002-1
- Food and Agriculture Organization of the United Nations/World Health Organization (2006). Probiotics in food: health and nutritional properties and guidelines for evaluation. *Food Agric. Organ. U. N.*
- Gianni de Carvalho, K., Gómez, J. E., Vallejo, M., Marguet, E. R., Peroti, N. I., Donato, M., et al. (2019). Production and properties of a bioemulsifier obtained from a lactic acid bacterium. *Ecotoxicol. Environ. Saf.* 183, 109553. doi:10.1016/j.ecoenv.2019.109553
- Goodacre, R., Vaidyanathan, S., Dunn, W. B., Harrigan, G. G., and Kell, D. B. (2004). Metabolomics by numbers: acquiring and understanding global metabolite data. *Trends Biotechnol.* 22, 245–252. doi:10.1016/j.tibtech.2004.03.007
- Habtegebriel, H., Wawire, M., and Sila, D. (2018). The effect of pretreatment (spray drying) on the yield and selected nutritional components of whole camel milk powder. *J. Food Sci.* 83 (12), 2983–2991. doi:10.1111/1750-3841.14361
- Hadaegh, F., Hasheminiya, M., Lotfaliany, M., Mohebi, R., Azizi, F., and Tohidi, M. (2013). Incidence of Metabolic syndrome over 9 years follow-up; the importance of sex differences in the role of insulin resistance and other risk factors. *PLoS One* 8, e76304. doi:10.1371/journal.pone.0076304
- Hasler, C. M. (2002). Issues and opinions functional foods: benefits, concerns and challenges—a position paper from the American Council on Science and Health 1. *J. Nutr.* 132, 3772–3781. doi:10.1093/jn/132.12.3772
- He, X., Cui, Y., Jia, Q., Zhuang, Y., Gu, Y., Fan, X., et al. (2025). Response mechanisms of lactic acid bacteria under environmental stress and their application in the food industry. *Food Biosci.* 64, 105938. doi:10.1016/j.fbio.2025.105938
- Ingelsson, E., Pencina, M. J., Tofler, G. H., Benjamin, E. J., Lanier, K. J., Jacques, P. F., et al. (2007). Multimarker approach to evaluate the incidence of the metabolic syndrome and longitudinal changes in metabolic risk factors: the framingham offspring study. *Circulation* 116, 984–992. doi:10.1161/CIRCULATIONAHA.107.708537
- Irie, K., Scott, A., and Hasegawa, N. (2014). Investigation of the detection ability of an intrinsic fluorescence-based bioaerosol detection system for heat-stressed bacteria. *PDA J. Pharm. Sci. Technol.* 68, 478–493. doi:10.5731/pdajpst.2014.01000
- Jonathan, I., Devanthi, P. V. P., Arham, A. G. A., Crystalia, A. A., Ying, C. L. S., and Pramanda, I. T. (2023). “Effects of temperature shock on viability and stress-related gene expression in *Pediococcus acidilactici*, a probiotic lactic acid bacteria,” in *IOP conference series: earth and environmental science* (Bali, Indonesia: Institute of Physics). doi:10.1088/1755-1315/1255/1/012068
- Katsui, N., Tsuchido, T., Hiramatsu, R., Fujikawa, S., Takano, M., and Shibasaki, I. (1982). Heat-induced blebbing and vesiculation of the outer membrane of *Escherichia coli*. *J. Bacteriol.* 151, 1523–1531. doi:10.1128/jb.151.3.1523-1531.1982
- Kim, D., Moon, J. S., Kim, J. E., Jang, Y. J., Choi, H. S., and Oh, I. (2024). Evaluation of purine-nucleoside degrading ability and *in vivo* uric acid lowering of *Streptococcus thermophilus* IDCC 2201, a novel antiuricemia strain. *PLoS One* 19, e0293378. doi:10.1371/journal.pone.0293378
- Książek, E. (2024). Citric acid: properties, microbial production, and applications in industries. *Molecules* 29, 22. doi:10.3390/molecules29010022
- Lee, A. K., Banta, A. B., Wei, J. H., Kiemle, D. J., Feng, J., Giner, J. L., et al. (2018). C-4 sterol demethylation enzymes distinguish bacterial and eukaryotic sterol synthesis. *Proc. Natl. Acad. Sci. U. S. A.* 115, 5884–5889. doi:10.1073/pnas.1802930115
- Li, Y., Chen, J., Feng, W., and Xiao, Y. (2025). Untargeted metabolomics and physiological phenotypic analyses reveal the defense strategies of nitrite by *Lactiplantibacillus plantarum* PK25. *Food Chem.* 463, 141338. doi:10.1016/j.foodchem.2024.141338
- Liao, Q., Hang, X., Liu, X., Pan, J., Zhang, H., and Yang, H. (2010). The influence of pH on heat stress response by probiotic *Lactobacillus plantarum* LP-Onlly. *Ann. Microbiol.* 60, 341–348. doi:10.1007/s13213-010-0048-x
- Liu, M., Zeng, X., He, Y., Xia, C., Cheng, L., Wu, Z., et al. (2021). iTRAQ-based quantitative proteomic analysis of the effect of heat shock on freeze-drying of *Lactobacillus acidophilus* ATCC4356. *Int. J. Food Sci. Technol.* 56, 5569–5580. doi:10.1111/ijfs.15101
- Liu, Z., Huang, X., Liu, Q., Yang, J., Li, J., Xiao, M., et al. (2025). Lactic acid bacteria fermentation improves sensory properties, bioactivity, and metabolic profiles of carrot pulp. *Food Biosci.* 66, 106303. doi:10.1016/j.fbio.2025.106303
- Machado, M. C., López, C. S., Heras, H., and Rivas, E. A. (2004). Osmotic response in *Lactobacillus casei* ATCC 393: biochemical and biophysical characteristics of membrane. *Arch. Biochem. Biophys.* 422, 61–70. doi:10.1016/j.jabb.2003.11.001
- Ou, C. C., Chiu, Y. H., Lin, S. L., Chang, Y. J., Huang, H. Y., and Lin, M. Y. (2012). Hepatoprotective effect of lactic acid bacteria in the attenuation of oxidative stress from tert-butyl hydroperoxide. *J. Food Drug Anal.* 20. doi:10.38212/2224-6614.2068
- Papadimitriou, K., Alegria, Á., Bron, P. A., de Angelis, M., Gobetti, M., Kleerebezem, M., et al. (2016). Stress physiology of lactic acid bacteria. *Microbiol. Mol. Biol. Rev.* 80, 837–890. doi:10.1128/mmb.00076-15
- Qian, Y., Ding, Q., Li, Y., Zou, Z., Yan, B., and Ou, L. (2014). Phosphorylation of uridine and cytidine by uridine–cytidine kinase. *J. Biotechnol.* 188, 81–87. doi:10.1016/j.jbiotec.2014.08.018
- Reimer, D., Cowles, K. N., Proschak, A., Nollmann, F. I., Dowling, A. J., Kaiser, M., et al. (2013). Rhabdopeptides as insect-specific virulence factors from entomopathogenic bacteria. *ChemBioChem* 14, 1991–1997. doi:10.1002/cbic.201300205
- Santivarangkna, C., Kulozik, U., and Foerst, P. (2008). Inactivation mechanisms of lactic acid starter cultures preserved by drying processes. *J. Appl. Microbiol.* 105, 1–13. doi:10.1111/j.1365-2672.2008.03744.x
- Schäfer, H., Beckert, B., Frese, C. K., Steinchen, W., Nuss, A. M., Beckstette, M., et al. (2020). The alarmones (p)ppGpp are part of the heat shock response of *Bacillus subtilis*. *PLoS Genet.* 16, e1008275. doi:10.1371/journal.pgen.1008275
- Siroli, L., Braschi, G., Rossi, S., Gottardi, D., Patrignani, F., and Lanciotti, R. (2020). *Lactobacillus paracasei* A13 and high-pressure homogenization stress response. *Microorganisms* 8, 439. doi:10.3390/microorganisms8030439
- Soukoulis, C., Behboudi-Jobbekdar, S., Yonekura, L., Parmenter, C., and Fisk, I. (2014). Impact of milk protein type on the viability and storage stability of microencapsulated *Lactobacillus acidophilus* NCIMB 701748 using spray drying. *Food Bioproc. Tech.* 7, 1255–1268. doi:10.1007/s11947-013-1120-x
- Sun, Y., Peng, C., Wang, J., Guo, S., Sun, Z. H., and Zhang, H. (2022). Mesopic fermentation contributes more to the formation of important flavor compounds and increased growth of *Lactobacillus casei* Zhang than does high temperature during milk fermentation and storage. *J. Dairy Sci.* 105, 4857–4867. doi:10.3168/jds.2021-20949
- Terpou, A., Papadaki, A., Lappa, I. K., Kachrimanidou, V., Bosnea, L. A., and Kopsahelis, N. (2019). Probiotics in food systems: significance and emerging strategies towards improved viability and delivery of enhanced beneficial value. *Nutrients* 11, 1591. doi:10.3390/nu11071591
- The Business Research Company (2024). *Probiotics-global-market-report*. London, United Kingdom: The Business Research Company. Available online at: <https://www.thebusinessresearchcompany.com/report/probiotics-global-market-report>.
- Timilsena, Y. P., Haque, Md. A., and Adhikari, B. (2020). Encapsulation in the food industry: a brief historical overview to recent developments. *Food Nutr. Sci.* 11, 481–508. doi:10.4236/fns.2020.116035
- Tripathi, M. K., and Giri, S. K. (2014). Probiotic functional foods: survival of probiotics during processing and storage. *J. Funct. Foods* 9, 225–241. doi:10.1016/j.jff.2014.04.030
- Walczak-Skierska, J., Zloch, M., Pauter, K., Pomastowski, P., and Buszewski, B. (2020). Lipidomic analysis of lactic acid bacteria strains by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry. *J. Dairy Sci.* 103, 11062–11078. doi:10.3168/jds.2020-18753
- Widodo, Handaka, R., Wahyuni, E., and Taufiq, T. T. (2017). The quality of fermented milk produced using intestinal-origin lactic acid bacteria as starters. *Int. Food Res. J.* 24, 2371–2376.
- Widodo, T., Taufik, T., Aryati, E., Kurniawati, A., and Asmara, W. (2012a). Human origin *Lactobacillus casei* isolated from Indonesian infants demonstrating potential characteristics as probiotics *in vitro*. *Indonesia J. Biotechnol.* 78.
- Widodo, S., Anindita, N., Tono Taufi, T., Dwi Wahyuningsih, T., and Asmara, W. (2012b). Identification of *Pediococcus* strains isolated from feces of Indonesian infants with *in vitro* capability to consume prebiotic inulin and to adhere on mucus. *Indones. J. Biotechnol.* 17, 132–143. doi:10.22146/ijbiotech.7852
- Widodo, W., Harsita, P. A., Sukarno, A. S., and Nurrochmad, A. (2019). Antidiabetic effect of milk fermented using intestinal probiotics. *Nutr. Food Sci.* 49, 1063–1074. doi:10.1108/NFS-11-2018-0326
- Widodo, W., Oktavisa Denta, A., Sunarti, S., and Haltrich, D. (2021). Milk fermented with *Lactobacillus casei* strain AP improves lipid profiles in obese Indonesian adults. doi:10.20944/preprints202108.0385.v1
- Widodo, W., Kusumaningrum, H. R. P., Wihadmyatami, H., and Wicaksana, A. L. (2023). Milk fermented with *Pediococcus acidilactici* strain BE improves high blood

glucose levels and pancreatic beta-cell function in diabetic rats. *Food Sci. Anim. Resour.* 43, 170–183. doi:10.5851/kosfa.2022.e69

Yang, H., He, M., and Wu, C. (2021). Cross protection of lactic acid bacteria during environmental stresses: stress responses and underlying mechanisms. *LWT* 144, 111203. doi:10.1016/j.lwt.2021.111203

Ye, Y., Zhang, L., Hao, F., Zhang, J., Wang, Y., and Tang, H. (2012). Global metabolomic responses of *Escherichia coli* to heat stress. *J. Proteome Res.* 11, 2559–2566. doi:10.1021/pr3000128

Zhang, Q., Zhao, C., Wang, X., Li, X., Zheng, Y., Song, J., et al. (2021). Bioaugmentation by *Pediococcus acidilactici* AAF1-5 improves the bacterial activity and diversity of cereal vinegar under solid-state fermentation. *Front. Microbiol.* 11, 603721. doi:10.3389/fmicb.2020.603721

Zhang, C., Han, Y., Gui, Y., Wa, Y., Chen, D., Huang, Y., et al. (2022). Influence of nitrogen sources on the tolerance of *Lactobacillus rhamnosus* to heat stress and oxidative stress. *J. Ind. Microbiol. Biotechnol.* 49, kuac020. doi:10.1093/jimb/kuac020

Zhen, N., Zeng, X., Wang, H., Yu, J., Pan, D., Wu, Z., et al. (2020). Effects of heat shock treatment on the survival rate of *Lactobacillus acidophilus* after freeze-drying. *Food Res. Int.* 136, 109507. doi:10.1016/j.foodres.2020.109507

Zheng, J., Wittouck, S., Salvetti, E., Franz, C. M. A. P., Harris, H. M. B., Mattarelli, P., et al. (2020). A taxonomic note on the genus *Lactobacillus*: description of 23 novel genera, emended description of the genus *Lactobacillus* Beijerinck 1901, and union of *Lactobacillaceae* and *Leuconostocaceae*. *Int. J. Syst. Evol. Microbiol.* 70, 2782–2858. doi:10.1099/ijsem.0.004107