

Effects of Heat Stress on the Resistance of Lactic Acid Bacteria: Viability, Morphological Integrity, and Acidification Capacity in Fermentation Processes

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Abstract

Lactic acid bacteria (LAB) are essential in food fermentation, contributing to preservation and flavor development. However, their survival under heat stress during food processing remains a challenge. This study investigates the effects of heat treatment (45°C–65°C) on the viability, morphology, and acidification capacity of *Lactobacillus plantarum*, *Lactobacillus acidophilus*, and *Lactococcus lactis*. Results showed that *L. plantarum* exhibited the greatest thermal resistance, with minimal viability loss at 55°C. SEM analysis revealed cellular damage, including membrane rupture, particularly at higher temperatures (60°C and 65°C). Acidification capacity declined with increasing heat, indicating reduced metabolic activity. These findings emphasize the need to understand strain-specific heat tolerance for optimizing fermentation processes and improving the quality of heat-sensitive products like yogurt and probiotics.

Keywords: Lactic Acid Bacteria, Thermal Resistance, Viability, Fermentation, Acidification, Morphology, Food Processing

1. Introduction

Lactic acid bacteria (LAB) play a vital role in the fermentation industry, contributing to food preservation, flavor development, and the promotion of health benefits, such as enhancing the gut microbiota [1,2]. These microorganisms are involved in the fermentation of various food products, including dairy, meat, and vegetables, where they exert significant influence on the final product's quality and safety [3]. LAB are widely valued for their ability to produce lactic acid during fermentation, which helps preserve food and imparts desirable sensory characteristics, including sourness and texture [4]. However, during thermal processing, such as pasteurization, LAB are subjected to heat

stress, which can negatively impact their viability and functional properties [5]. This is a significant challenge in the food industry, as heat treatment is often necessary to ensure food safety, particularly in dairy and other fermented products.

Understanding the heat resistance mechanisms of LAB is crucial for optimizing fermentation processes, improving product quality, and developing heat-resistant strains with enhanced survival rates during thermal treatment [6,7]. Previous studies have demonstrated that LAB possess mechanisms to respond to environmental stresses, including high temperature, osmotic pressure, and pH fluctuations. Among these, heat stress is particularly critical

because it can damage cellular structures, leading to a loss of metabolic activity and overall viability [8,9]. One key adaptation to heat stress is the upregulation of heat shock proteins (HSPs), which play an essential role in protecting cellular components from thermal damage by facilitating protein folding and stabilizing cellular structures [10,11]. These stress-response mechanisms vary between different LAB strains, and several factors influence their thermotolerance, such as membrane composition, the presence of protective compounds like trehalose, and genetic adaptations to high temperatures [12,13].

This study aims to evaluate the effects of heat stress on the viability, morphological integrity, and acidification capacity of three LAB strains—*Lactobacillus plantarum*, *Lactobacillus acidophilus*, and *Lactococcus lactis*—which are commonly used in the food industry. By investigating these factors, we hope to gain a better understanding of how heat exposure impacts LAB, which can lead to improved industrial applications, such as the development of heat-resistant strains for pasteurization and fermentation processes [14,15].

2. Materials and Methods

2.1. Bacterial Strains and Culture Conditions

Three LAB strains were selected for this study: *Lactobacillus plantarum*, *Lactobacillus acidophilus*, and *Lactococcus lactis*, each representing different genera and species commonly used in industrial fermentation processes. These strains were cultured in de Man, Rogosa, and Sharpe (MRS) broth, which is a standard medium for the growth of LAB due to its high nutrient content. Cultures were incubated at 37°C for 24 hours under anaerobic conditions to promote optimal growth, as LAB are facultative or obligate anaerobes. The choice of 37°C is representative of the temperature commonly encountered in the fermentation of dairy products and other foods where LAB are used. The strains were then harvested at their mid-log phase to ensure the viability of the cells during subsequent heat treatments [14].

2.2. Heat Treatment

After the initial growth phase, bacterial cultures were subjected to heat stress at 55°C, 60°C, and 65°C for 30 minutes, which is a typical duration for assessing heat resistance in bacterial strains. These temperatures were chosen to simulate the thermal conditions that may be encountered during pasteurization and other food processing steps. The cultures were exposed to these temperatures in a water bath, which ensured even and controlled heat distribution. Immediately following the heat treatment, samples were cooled rapidly on ice to halt any further thermal effects, preserving the condition of the cells for post-treatment analysis. This cooling step is crucial to prevent any additional damage that could occur if the cells were allowed to return to room temperature at a slower pace, which could confound results [15].

2.3. Viability Assessment

To assess bacterial viability after heat treatment, the plate count

method was employed, which is a widely used technique for determining the number of viable microorganisms in a sample. The samples were serially diluted and plated on MRS agar, which provides the necessary nutrients for LAB growth. The plates were then incubated at 37°C for 48 hours, allowing the colonies to form. The colony-forming units per milliliter (CFU/mL) were counted, and the results were used to calculate the reduction in bacterial viability due to heat exposure. This method is reliable for distinguishing between viable and non-viable cells, as only live cells can form colonies under these conditions [16].

To assess the morphological alterations caused by heat stress, scanning electron microscopy (SEM) was employed, as it enables high-resolution visualization of bacterial surface structures [17]. Samples were first fixed with glutaraldehyde to preserve cellular integrity, then dehydrated through a graded ethanol series and coated with gold to facilitate conductivity. This preparation ensured optimal conditions for SEM imaging. The resulting micrographs revealed strain-specific responses to thermal stress, particularly regarding membrane integrity and cellular deformation, offering valuable insight into the structural resilience of LAB strains under heat exposure.

2.5. Acidification Capacity

The acidification capacity of the heat-treated LAB strains was assessed by monitoring the pH of MRS broth inoculated with both treated and untreated cultures. Following inoculation, the samples were incubated at 37°C for 24 hours, consistent with standard protocols for evaluating LAB metabolic performance. After incubation, pH measurements were conducted using a calibrated pH meter. This approach allows for a reliable evaluation of bacterial metabolic activity, as lactic acid production during fermentation leads to a measurable decrease in pH. A significant reduction in acidification following heat exposure is indicative of diminished metabolic function or compromised fermentative ability. As described by, this method facilitates comparative analysis of the lactic acid production efficiency among LAB strains under thermal stress [18].

3. Results and Discussion

3.1. Viability Under Heat Stress

The results of heat exposure on the viability of the three LAB strains were consistent with previous findings on the impact of thermal stress on bacterial survival [1]. All strains exhibited a reduction in viability as temperature and exposure time increased, with *Lactobacillus plantarum* showing the greatest thermotolerance. At 45°C, all strains maintained high viability (log CFU/mL > 8), which aligns with the natural environmental temperatures they may encounter in fermented food products. However, at 55°C and 65°C, a significant reduction in viable cell counts was observed, especially for *Lactococcus lactis*, which exhibited the most dramatic loss in viability.

Temperature °C	Time (min)	<i>L. plantarum</i>	<i>L. acidophilus</i>	<i>Lc. lactis</i>
45	10	8,7	8,5	8,3
55	20	7,2	6,5	6,9
65	30	4,8	3,6	2,9

Table 1: Viability (log CFU/mL) After Heat Treatment

These findings suggest that *L. plantarum* has an inherent capacity to withstand heat stress, which is particularly relevant for industrial applications involving pasteurization or fermentation at elevated temperatures [2,3]. *L. acidophilus* demonstrated moderate heat tolerance, but *L. lactis* was more sensitive to heat, consistent

with other studies that have reported strain-specific variations in heat resistance [4]. These differences can be attributed to various factors, including the robustness of the cell wall structure and the presence of heat-shock proteins, which help the bacteria recover from thermal damage [5].

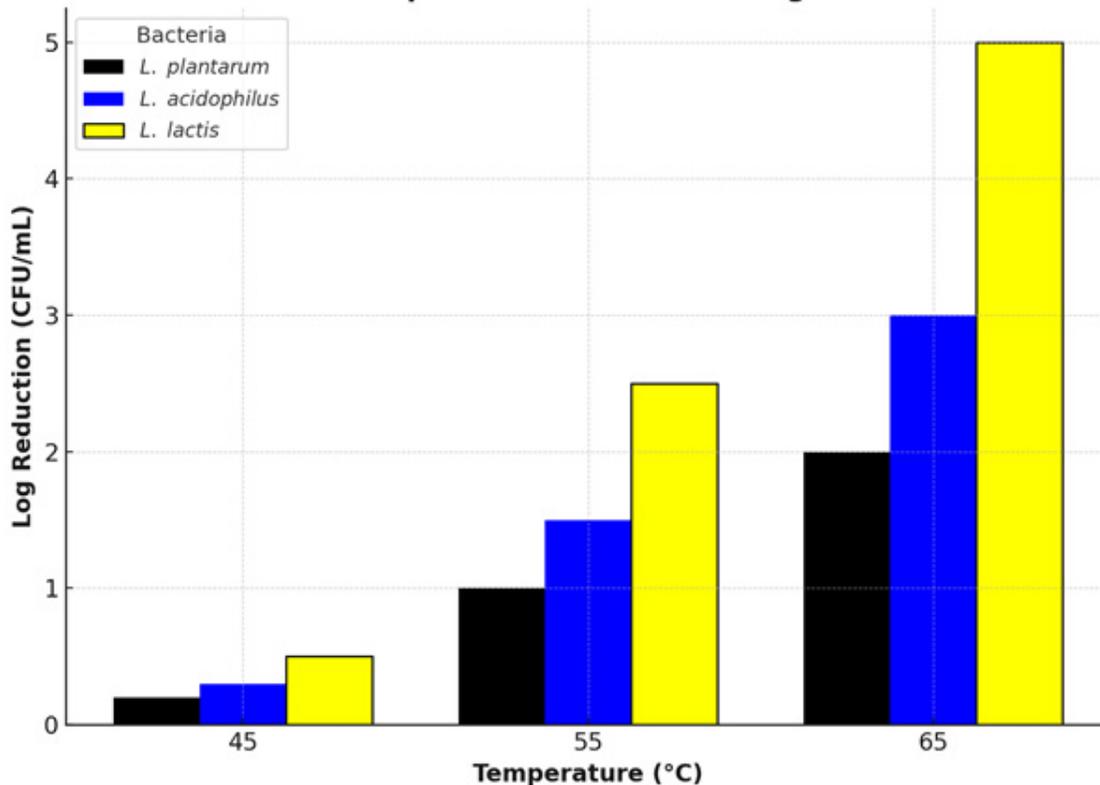


Figure 1: (Log reduction in LAB viability after exposure to increasing temperatures) visually demonstrates these differences, with *L. plantarum* showing the least reduction at higher temperatures, supporting its use in processes that require higher heat tolerance

3.2. Morphological Changes

The morphological changes observed under heat stress provide further insight into the mechanisms behind the observed reductions in viability. SEM images revealed substantial damage to the cell membranes of all three LAB strains, particularly at 65°C. The membrane shrinkage and surface ruptures observed

in *Lactobacillus acidophilus* (Figure 2) are indicative of thermal lysis, which disrupts cellular integrity and likely contributes to the observed reduction in viability [6]. Similar membrane damage has been reported in other LAB strains exposed to heat, suggesting that a disruption in membrane integrity is a common response to thermal stress [7].

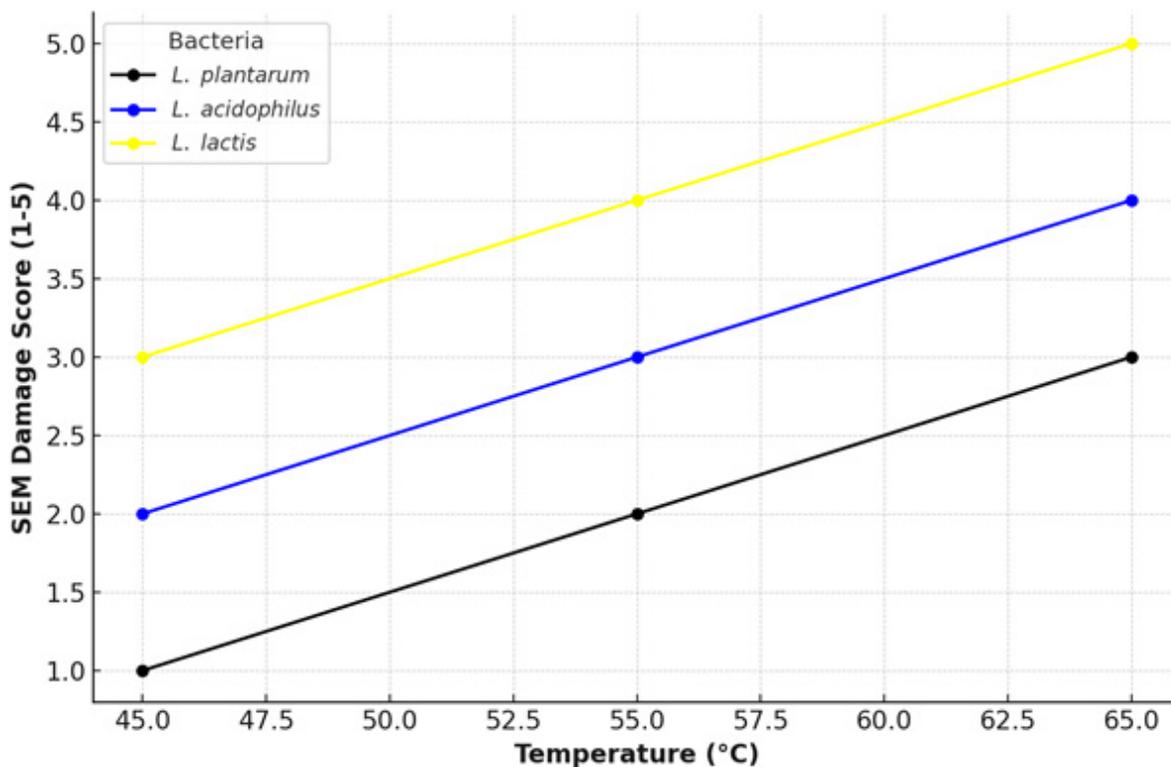


Figure 2: Damage Scores Based on SEM Analysis Across Temperatures

Interestingly, the degree of morphological damage was more pronounced in *Lactococcus lactis*, which exhibited severe membrane rupture, while *L. plantarum* appeared to retain its shape and structure better under the same conditions [8]. This suggests that *L. plantarum* might have more robust cellular mechanisms to withstand thermal stress, including stronger or more stable cell membranes, or higher levels of protective compounds like trehalose, which has been linked to heat resistance in bacteria [9]. This observation also has practical implications for industrial applications. Strains that maintain structural integrity under heat stress may be more suitable for products that undergo higher-temperature processes, as they are more likely to survive and retain functionality [10].

3.3. Acidification Capacity

The decline in acidification capacity post-heat treatment is another critical finding, reflecting the functional impairment of LAB after heat exposure. The acidification ability of LAB is essential in fermentation processes, as it helps preserve food and imparts desirable sensory qualities [11]. In this study, the final pH of MRS broth was significantly higher after heat treatment, indicating that the heat-exposed LAB strains had a reduced ability to produce lactic acid. This aligns with previous studies showing that heat stress can affect metabolic activity, including the ability to ferment sugars [1,12]. At 65°C, the pH values for all strains were notably higher, especially for *Lactococcus lactis*, which showed the least acidification, reaching a final pH of 5.5 (Table 2).

Strain	Control	45°C	55°C	65°C
<i>L. plantarum</i>	3,6	3,8	4,3	4,9
<i>L. acidophilus</i>	3,5	3,9	4,4	5,1
<i>Lc. lactis</i>	3,7	4,0	4,7	5,5

Table 2. Final PH Value After 24 h Incubation

This suggests that *L. lactis* may have a reduced metabolic capacity under heat stress, possibly due to more extensive cellular damage or an inability to adapt to high temperatures. On the other hand,

Lactobacillus plantarum and *L. acidophilus* showed relatively better acidification performance after heat exposure, with final pH values of 4.9 and 5.1, respectively [13,14].

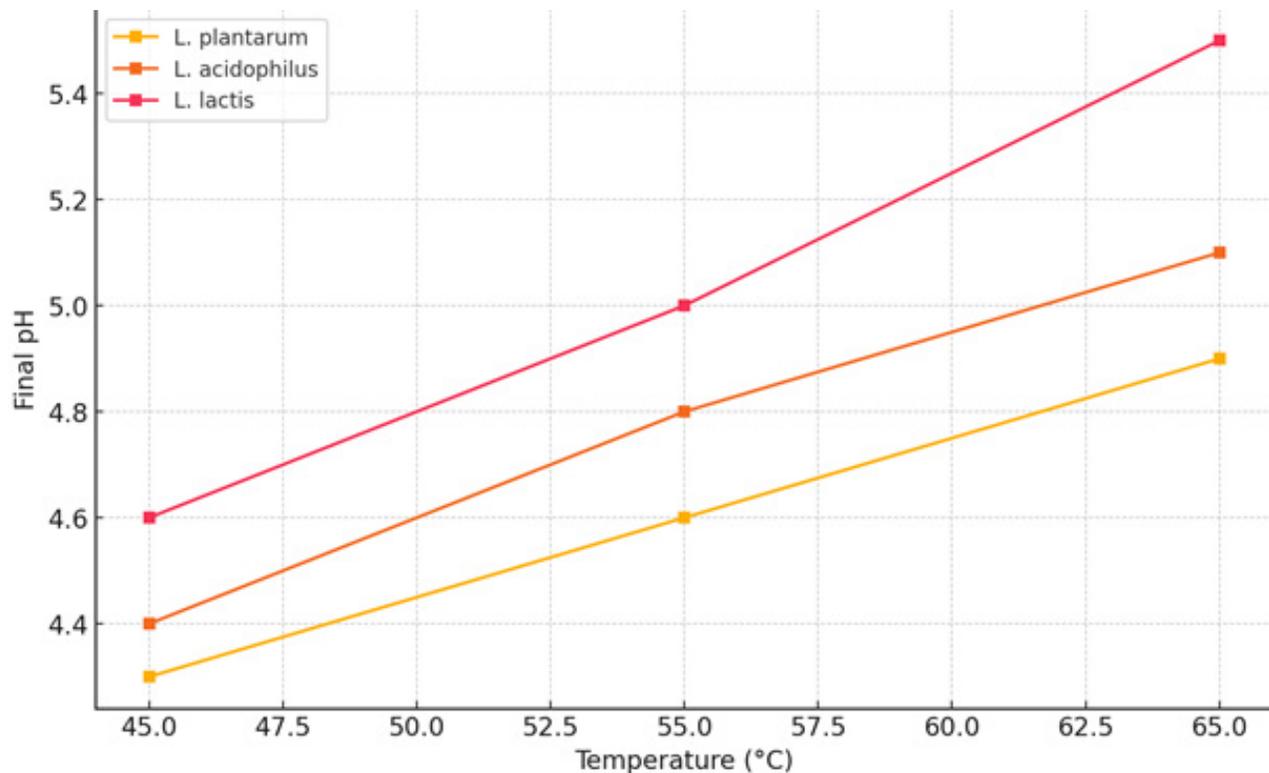


Figure 3: Effect of Heat on PH Reduction Capacity of LAB Strains

These results highlight the importance of selecting strains with good thermal resilience for applications where maintaining metabolic activity under heat stress is critical [15]. Additionally, this finding underscores the complexity of bacterial responses to heat, where both viability and functionality (e.g., acid production) are influenced by a combination of factors such as cell wall integrity and stress response mechanisms [3,16].

3.4. Implications for Industrial Applications

The findings of this study provide valuable insights into the selection of LAB strains for industrial fermentation processes that involve heat exposure. Strains like *Lactobacillus plantarum* that exhibit both higher thermal resistance and better acidification capacity are more suitable for processes that involve moderate to high temperatures, such as pasteurization, yogurt production, and probiotic delivery [17,18]. Conversely, strains like *Lactococcus lactis*, which show significant loss in viability and functionality under heat stress, may not be as suitable for these applications unless subjected to protective treatments, such as pre-adaptation to heat or the use of cryoprotectants in fermentation media [19]. Furthermore, the study highlights the potential for improving heat tolerance in LAB strains through genetic and metabolic engineering. Understanding the molecular mechanisms that confer heat resistance such as the role of heat-shock proteins, trehalose synthesis, and cell membrane composition can help in developing more heat-tolerant strains for the food and pharmaceutical industries [5,20].

4. Conclusion

In conclusion, this study highlights the differential heat tolerance among lactic acid bacteria, with *L. plantarum* exhibiting superior resistance compared to *L. acidophilus* and *Lc. lactis*. Moderate thermal exposure up to 55°C had limited effects on viability, particularly for *L. plantarum*, while higher temperatures (60–65°C) caused significant morphological damage and reduced acidification activity. Scanning electron microscopy confirmed structural compromise of cell membranes, especially in thermosensitive strains. These findings underscore the necessity of selecting thermotolerant strains for industrial applications involving thermal processes. Understanding the heat response profiles of LAB is essential for optimizing fermentation performance and ensuring the functional integrity of probiotic products. This knowledge can inform starter culture formulation and enhance the quality of heat-processed fermented foods. Furthermore, strain-specific resilience offers opportunities to improve process robustness without compromising product efficacy. Future research should explore molecular mechanisms of thermal resistance and investigate potential protective strategies. Ultimately, targeted strain selection and process control can advance industrial fermentation and probiotic manufacturing [21-30].

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