71st ICoMST
GIRONA - CATALONIA - SPAIN
3rd-8th August - 2025

COLD-STRESS-INDUCED EXTRACELLULAR METABOLITE SHIFTS IN *L. MONOCYTOGENES* IN CHICKEN SIMULATING BROTH

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I. INTRODUCTION

Listeria monocytogenes is a psychrotrophic foodborne pathogen capable of surviving and proliferating at refrigeration temperatures (0-10°C). This characteristic poses a significant threat to food safety, particularly in chilled meat products, where *L. monocytogenes* can grow during storage without causing noticeable spoilage. Previous studies have primarily used synthetic culture media such as Tryptic Soy Broth or Brain Heart Infusion to investigate the physiology and metabolism of *L. monocytogenes* under various environmental stresses [1]. Although these media offer high reproducibility, they may not accurately replicate the complex nutritional environment of meat. To overcome this limitation, we developed a chicken-derived culture medium that simulates the composition of meat [2]. In this study, we aimed to investigate the cold stress response of *L. monocytogenes* using the meat-simulating culture medium.

II. MATERIALS AND METHODS

Three *L. monocytogenes* strains (NCCP 15143, NCCP 16594, and ATCC 19111) were used. The bacterial suspensions were inoculated into a chicken-derived medium (approximately 1 log CFU/mL), prepared as described by Kim et al. [2]. Cultures were incubated at 8°C for 22 days, and samples were collected every 2 days (from day 0 to day 22). Bacterial growth was monitored by plating serial dilutions on XLD agar and incubating at 37°C for 24 h (CFU/mL). Extracellular metabolites were collected by centrifugation (12,000×g, 20 min, 4°C), followed by filtration (0.22 μm PTFE syringe filter). Metabolites were analyzed using nuclear magnetic resonance spectroscopy (1D ^{1}H-zg30). Metabolite data were normalized by log transformation and autoscaling. Heatmap was performed using MetaboAnalyst 5.0. Metabolic pathway analysis was conducted using the KEGG database. Significant differences in bacterial growth or metabolite concentrations were evaluated using one-way ANOVA followed by Tukey's post hoc test, with a significance level set at $P < 0.05$.

III. RESULTS AND DISCUSSION

The growth patterns of Listeria monocytogenes strains in the chicken-derived culture medium are shown in Figure 1. The initial inoculation levels of Listeria monocytogenes in the chicken-derived culture medium were 1.18, 1.15, and 1.16 log CFU/mL for NCCP 15143 (L5), NCCP 16594 (L6), and ATCC 19111 (L9), respectively.

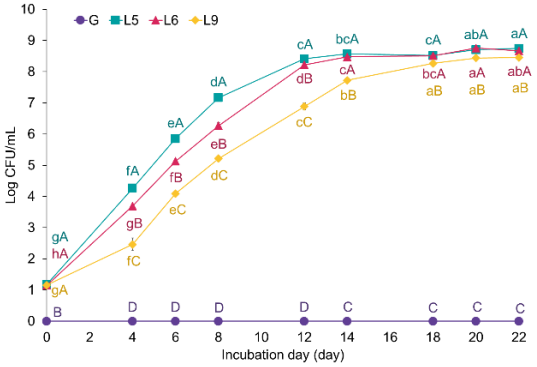


Figure 1. Growth of *Listeria monocytogenes* strains in chicken-derived culture medium at 8°C. G, non-inoculated control; L5, *L. monocytogenes* NCCP 15143; L6, NCCP 16594; L9, ATCC 19111.

No significant differences in bacterial counts were observed on the day of inoculation. L5 showed significantly higher counts than L6 and L9 up to day 12. L6 also exhibited significantly higher growth than L9 during this period. From day 14, no significant difference was observed between L5 and L6. All three strains reached the stationary phase at approximately 8 log CFU/mL. The stationary phase was reached on day 12 for L5 and L6, and on day 18 for L9. No microbial growth was detected in the uninoculated culture medium throughout the entire incubation period.

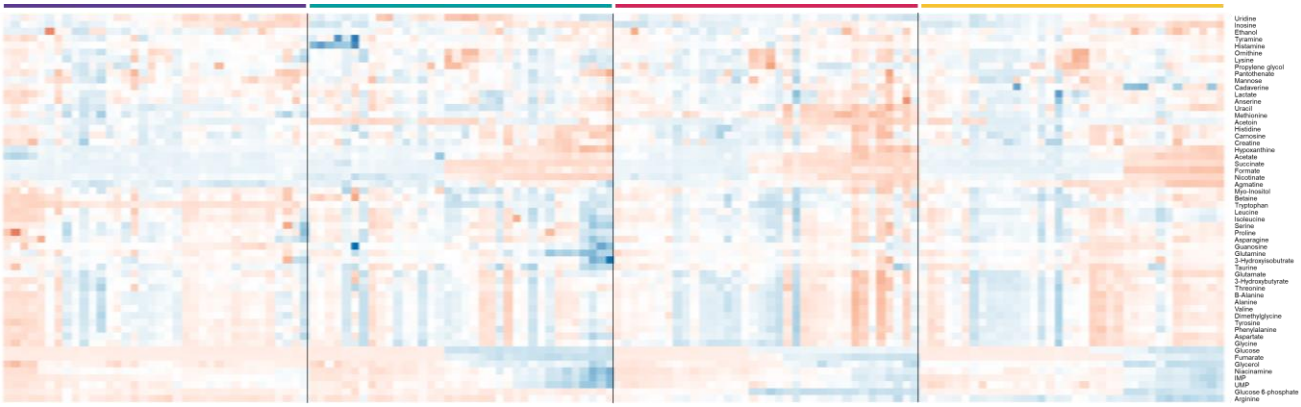


Figure 2. Heatmap of extracellular metabolite profiles of *Listeria monocytogenes* strains incubated at 8°C in chicken-derived culture medium. Purple, non-inoculated control; Green, NCCP 15143 (L5); Red, NCCP 16594 (L6); Yellow, ATCC 19111 (L9).

Heatmap analysis revealed distinct differences in extracellular metabolite profiles between the non-inoculated control and the inoculated broths (L5, L6, and L9) (Figure 2). In the control group, metabolite levels remained stable throughout the incubation period. In contrast, all three *L. monocytogenes* strains showed time-dependent alterations in their extracellular metabolite profiles. Common metabolic patterns were observed across the inoculated groups. Specifically, hypoxanthine, acetate, formate, nicotinate, and agmatine showed a time-dependent increase, while glucose, fumarate, glycerol, nicotinamide, IMP, UMP, glucose 6-phosphate, and arginine decreased over time relative to the control. Notably, these changes became visually apparent in the heatmap starting from day 12 of incubation for strains L5 and L6, and from day 18 for strain L9, as reflected by a clear transition in color intensity.
The time-dependent alterations in extracellular metabolites suggest a metabolic response during bacterial growth. Metabolites that increased over time are primarily associated with purine metabolism, pyruvate metabolism, and pathways related to stress response and polyamine biosynthesis. Hypoxanthine reflects purine degradation, while acetate and formate are end-products of fermentative energy metabolism. The increase in nicotinate and agmatine may indicate an enhanced bacterial stress response and cellular adaptation during prolonged incubation. In contrast, the metabolites that decreased over time are central to glycolysis, the TCA cycle, and nucleotide metabolism. Their depletion implies active utilization to support energy generation and macromolecular biosynthesis. The reduced levels of IMP and UMP reflect their involvement in nucleic acid synthesis during bacterial replication.

IV. CONCLUSION

The results of this study showed that Listeria monocytogenes undergoes metabolic changes to survive and grow under low-temperature conditions. The chicken-derived culture medium serves as a useful model for studying bacterial physiology in meat-like environments. This study offers a novel approach to examining the cold stress responses of microorganisms in the meat industry, and the identified metabolites may serve as predictive markers to improve food safety management during refrigerated storage.

ACKNOWLEDGEMENTS

This work was carried out with the support of the National Research Foundation of Korea (NRF) grant funded by the Korea government (MSIT) (No. RS-2024-00453016).

REFERENCES

1. Kim, H.; Kim, H; Hong, H.; Jo, C. (2022) Metabolomic approaches for the detection of Listeria monocytogenes and Staphylococcus aureus in culture media. LWT 171: 114117.
2. Kim, H.; Kim, H; Hong, H.; Jo, C. (2024) Meat-derived broth as a novel culture medium for metabolomic study of bacteria in meat. LWT 201: 116254.