Listeria monocytogenes persister cell formation triggered upon antimicrobial exposure

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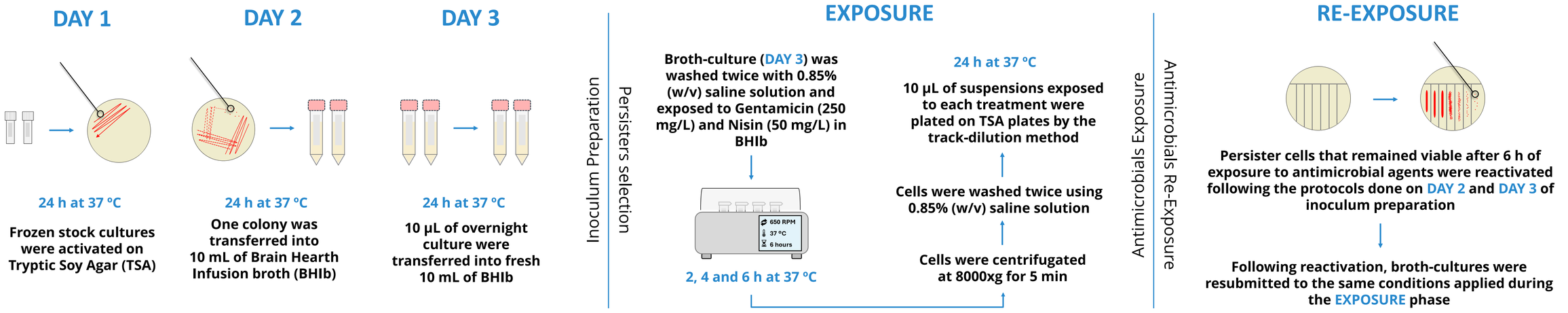
Introduction

Listeria monocytogenes, responsible for listeriosis, is a significant foodborne pathogen. Listeriosis poses a serious health risk, especially for vulnerable populations such as the elderly, immunocompromised individuals, pregnant women and infants. The pathogen's ability to survive and grow under harsh conditions, including refrigeration temperatures and high concentrations of salt or disinfectants, enables its persistence within food processing environments, posing a considerable risk to consumers. One of the potential mechanisms used by *L. monocytogenes* to withstand such unfavorable conditions is the formation of persister cells: a subpopulation of growth-arrested cells capable of transiently tolerating bactericidal levels of antimicrobial agents. This state can be triggered by exposure to high concentrations of stress-inducing compounds (e.g., antibiotics). Once conditions become favorable again, these cells can resume normal metabolic activity.

Objectives

This study aimed to investigate the formation of persister cells in two *L. monocytogenes* strains (Lm 1712 and Lm 2116) previously characterized and isolated from food production environments (FPE). These cultures were kindly provided by the *GenoPhenoTraits4Persistence* project culture collection for this study.

Methods



Results

Listeria monocytogenes

GenoPhenoTraits4Persistence Project

Strain	Lm 1712	Lm 2116
Origin	Dairy FPE	Dairy FPE
Persistent	No	Yes
Lineage	II	I
Clonal Complex (CC)	CC 155	CC 1
Sequence Type (ST)	ST 155	ST 1

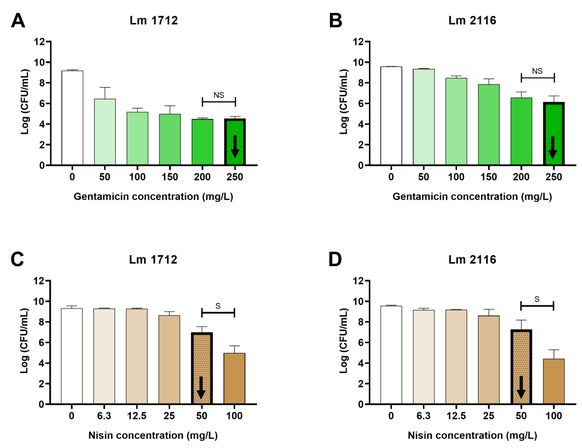


Figure 1 – *Listeria monocytogenes* exposure to increasing concentrations of Gentamicin (A and B) and Nisin (C and D) for 4 hours at 37 °C (650 RPM). Final concentration chosen for exposure and re-exposure treatment is denoted with a patterned bar [⬇]. Significant (N) and No Significant (NS) differences between conditions were assessed through ANOVA using Tukey's multiple comparisons test ($p<0.05$). All values at each concentration tested are represented as Log(CFU/mL) means ± SD (n=3).

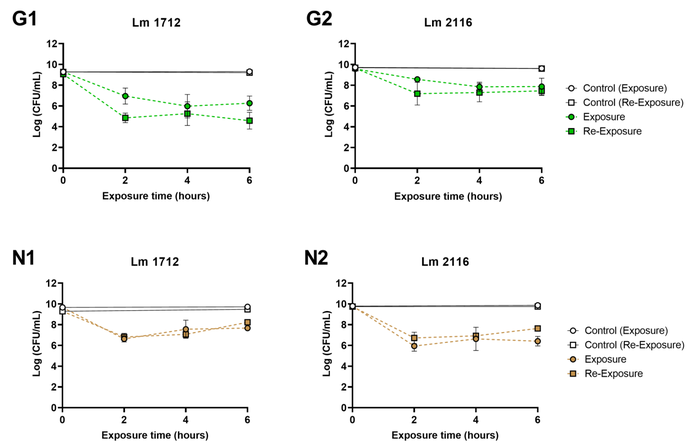


Figure 2 – *Listeria monocytogenes* exposure and re-exposure to Gentamicin at 250 mg/L (G1 and G2) and Nisin at 50 mg/L (N1 and N2) was performed for a total time of 6 hours at 37 °C (650 RPM). All values at each timepoint tested are represented as Log(CFU/mL) means ± SD (n=3).

Conclusions

- Both strains are capable of triggering persister cell formation upon exposure to high concentrations of either antimicrobial, as previously reported for *L. monocytogenes* exposed to these agents[1,2].
- increasing levels of antimicrobial agents, such as gentamicin and nisin, can induce persister cell formation once a threshold concentration is reached. Based on our data, 250 mg/L, the point at which the biphasic killing curve plateaued, represents the optimal concentration for gentamicin. For nisin, given its membrane-damaging activity, 50 mg/L was selected as the target concentration to evaluate the behavior of both strains in this study.
- After six hours of exposure to gentamicin, the total bacterial population (log CFU/mL) decreased from 9.3 to 6.3 for Lm 1712 and from 9.7 to 7.9 for Lm 2116. A similar trend was observed for nisin, with counts decreasing from 9.7 to 7.7 (Lm 1712) and from 9.8 to 6.4 (Lm 2116). Upon re-exposure, both strains displayed killing curves comparable to those observed during the initial exposure, indicating the selection of *L. monocytogenes* persister cells rather than a resistant subpopulation.
- The two strains responded differently to the antimicrobial agents. Notably, a higher tolerance to gentamicin after six hours, as observed in Lm 2116, did not confer a similar capacity against nisin, suggesting strain-specific differences in their responses.
- Future studies should assess the effects of gentamicin and nisin at concentrations higher than those tested here, as well as longer exposure times (>6 h), to further characterize persister cell tolerance under these conditions.
- The potential impact of alternative antimicrobial agents, such as benzalkonium chloride (BAC) or peracetic acid (PAA), should also be explored in subsequent work.

Acknowledgements

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References

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