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Cell-free supernatants from foodborne lactic acid bacteria isolates disrupt the AI-2 bacterial quorum sensing system and inhibit biofilm formation in monocultures of *Listeria monocytogenes* and *Staphylococcus aureus*

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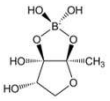
INTRODUCTION & AIM

- Biofilms composed of foodborne bacterial pathogens pose a significant challenge to food safety as they enhance microbial persistence in processing environments.
- The autoinducer-2 (AI-2) quorum sensing (QS) signaling system is widely conserved among both Gram-positive and Gram-negative bacteria and is involved in both intra- and inter-species communication.
- This is employed to regulate group behaviors based on cell density and plays a crucial role in biofilm formation by pathogens such as *L. monocytogenes* and *S. aureus*.
- Lactic acid bacteria (LAB) have been used for centuries in food fermentation to improve sensory and nutritional profiles and preserve against detrimental microflora.
- The use of LAB and/or their metabolites as natural quorum-sensing inhibitors (QSIs) may represent a promising, eco-friendly antibiofilm strategy.
- **This study investigates the ability of LAB-derived cell-free supernatants (CFSs) to interfere with AI-2-mediated QS and inhibit biofilm formation in monocultures of *L. monocytogenes* and *S. aureus*.**

METHODS

1 LAB Supernatant Preparation
89 LAB isolates cultured, centrifuged, neutralized, and filtered to obtain CFSs.

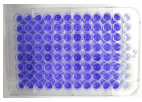
2 AI-2 Activity Screening
Vibrio harveyi bioluminescence assays used to detect AI-2-like signals and QS inhibition.



autoinducer-2 (AI-2)

3 Determintation of MIC
Broth microdilution method to determine the MICs of 20 selected LAB CFSs against the two pathogens.

4 Biofilm Formation Testing
Microtiter plate biofilm assays conducted with 20 selected LAB CFSs at sub-MIC level (50% v/v).



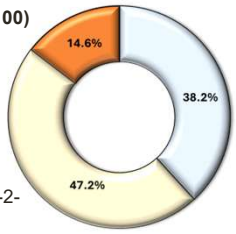
5 Growth Kinetics Analysis
Planktonic growth parameters determined to distinguish between growth inhibition and specific anti-biofilm effects.

RESULTS & DISCUSSION

AI-2-like Activity in LAB CFSs

Strong Signal (RLU > 100)
14.6% of LAB CFSs
Produced high levels of AI-2-like molecules

Moderate Signal (RLU 10-100)
47.2% of LAB CFSs
Contained detectable AI-2-like signals



Low/No Signal (RLU < 10)
38.2% of LAB CFSs
Minimal AI-2-like activity detected

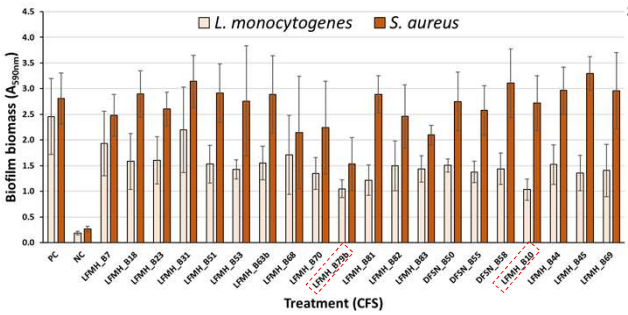
RLU < 10
10 ≤ RLU ≤ 100
RLU > 100

AI-2 Quorum Sensing Inhibition (QSI) of LAB CFSs

28.1%
Strong Inhibitors
LAB CFSs showing ≥90% inhibition of AI-2 QS activity

25
Potent Inhibitors
Number of isolates demonstrating robust AI-2 QSI

Anti-Biofilm Effects of LAB CFSs Applied at sub-MIC (50% v/v)



→ 90% of 20 LAB CFSs with AI-2 QS interference significantly reduced *L. monocytogenes* biofilm biomass, and one (LFMH_B79b) also decreased that of *S. aureus* by 45.4%.

Specific Anti-Biofilm Action
Most LAB CFSs did not significantly affect planktonic growth parameters of the two pathogens.

This suggests a biofilm-specific inhibition mechanism rather than general antimicrobial activity.

KEY FINDINGS & CONCLUSIONS

1. **AI-2 Signal Production**
61.8% of LAB CFSs contained AI-2-like signals that could induce luminescence in reporter strain.
2. **Biofilm Inhibition**
90% of tested LAB CFSs significantly reduced *L. monocytogenes* biofilm formation without affecting planktonic growth.
3. **Species-Specific Effects**
S. aureus biofilms were more recalcitrant, with only one LAB CFS showing significant inhibition.
4. **Most Effective LAB Strains**
The most effective anti-biofilm agents were derived from *Enterococcus faecium* **LFMH_B79b** and *Pediococcus acidilactici* **LFMH_B10**, reducing *L. monocytogenes* biofilm biomass by over 57%.

→ LAB metabolites may offer a sustainable, non-toxic approach to controlling foodborne pathogen biofilms.

→ Sub-inhibitory concentrations minimize selective pressure on bacteria.

FUTURE WORK

1. **Compound Identification**
Isolate and characterize specific anti-biofilm molecules from LAB CFSs.
2. **Mechanism Studies**
Investigate molecular pathways through transcriptomic and proteomic analyses.
3. **Multi-Species Biofilms**
Test effectiveness against complex, mixed-species biofilms.
4. **Real-World Applications**
Evaluate performance under actual food processing conditions.

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