

# Context matters: environmental microbiota from ice cream processing facilities affected the inhibitory performance of two lactic acid bacteria strains against *Listeria monocytogenes*

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**ABSTRACT** Using biocontrol microorganisms to complement sanitation of dairy processing facilities may enhance the control of pathogenic *Listeria monocytogenes*. However, it remains unknown whether the microbiota of dairy processing facilities affects the antilisterial activity of biocontrol strains. We tested two lactic acid bacteria (LAB) strains (*Enterococcus faecium* PS01155 and *Enterococcus lactis* PS01156) for their antilisterial potential in the context of microbiomes collected from three ice cream processing facilities (A, B, and C). Antilisterial ability was assessed by co-culturing LAB strains with eight *L. monocytogenes* strains in the presence of microbiota for 3 days at 15°C, followed by quantification of the most probable number of attached *L. monocytogenes*. We observed no significant reduction of the *L. monocytogenes* concentration in the samples co-cultured with LAB strains in the presence of environmental microbiota collected from ice cream processing facilities, compared to a positive control containing only the *L. monocytogenes* cocktail and the environmental microbiomes. The attachment of LAB strains to an abiotic surface was assessed by co-culturing LABs with microbiomes at 15°C for 3 days, followed by characterization of attached microbiota composition using amplicon sequencing. All treatment samples that contained microbiomes from dairy processing facilities had a high relative abundance of *Pseudomonas*. Further, the presence of *Pseudomonas* isolated from the microbiome from facility B significantly reduced the antilisterial ability of *E. faecium* PS01155.

**IMPORTANCE** Antilisterial LAB strains have been proposed as biological control agents for application in food processing environments. However, the effect of resident food processing environment microbiota on the performance on antilisterial LAB strains is poorly understood. Our study shows that the presence of microbiota collected from ice cream processing facilities' environmental surfaces can affect the attachment and inhibitory effect of LAB strains against *L. monocytogenes*. Further studies are therefore needed to assess whether individual microbial taxa affect antilisterial properties of LAB strains and to characterize the underlying mechanisms.

**KEYWORDS** ice cream, food processing facilities, environmental microbiomes, *Listeria monocytogenes*, biocontrol, protective cultures, lactic acid bacteria, *Enterococcus*

The foodborne pathogen *Listeria monocytogenes* causes listeriosis, a deadly foodborne illness. In the United States, the Center for Disease Control and Prevention (CDC) reported 80 outbreaks of listeriosis between 2009 and 2020, which resulted in 818 illnesses, 705 hospitalizations, and 128 deaths (1). Listeriosis outbreaks have been linked to the consumption of dairy foods, including raw milk, cheese, and ice cream (2). In 2015–2016, a multistate outbreak of listeriosis traced to ice cream products served in hospital settings (3) revealed that *L. monocytogenes* can survive in frozen dairy products (4). While *L.*

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