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Assessment of Optimal Cultivation and Antibacterial Property of *Azolla* Fermented with Lactic Acid Bacteria

Sarah Yasmin Shahidi^a, Farizan Aris^{a*}

Structured Abstract

Background: Malaysia confronts significant challenges in poultry farming, particularly with broilers' susceptibility to various bacteria, notably *Staphylococcus aureus*. The rise of antimicrobial-resistant *S. aureus* strains among animal handlers underscores the urgent need for antibiotic-free practices. Research suggests that *Azolla*, a prolific freshwater fern known for its rapid growth and rich protein content, can provide both nutritional benefits and antibacterial properties when incorporated into poultry feed, making it a potential sustainable alternative to antibiotics. However, optimal cultivation conditions for *Azolla* in Malaysia's climate are not well understood, hampering its effective use. Additionally, the combination of fermented *Azolla* with lactic acid bacteria (LAB), such as *Lactobacillus casei*, and its antibacterial effects against *S. aureus* remain unexplored. LAB is recognized for its probiotic properties and benefits on gut health, which can enhance *Azolla*'s nutritional composition and antibacterial properties when used in solid-state fermentation (SSF). Identifying the optimal conditions for *Azolla* cultivation in Malaysia and investigating its fermentation with LAB could significantly contribute to sustainable and nutritious feed sources for poultry, addressing both feed efficiency and antibacterial concerns. This study aims to analyze the growth curve of LAB isolated from probiotic drinks and investigate the effects of SSF on *Azolla*'s antibacterial properties against *S. aureus*.

Methods: The SSF of *Azolla* with isolated LAB was performed for 72 hours. The antibacterial property of fermented *Azolla* towards *S. aureus* was analysed through agar well and disc diffusion assay and further confirmed with a minimum inhibitory concentration (MIC) test.

Results: This study shows that LAB exhibited a 3-hour lag phase, rapid exponential growth from 3-6 hours, peak growth at 26 hours, and a slight decline from 27-29 hours. Unfermented *Azolla* showed moderate antibacterial activity against *S. aureus*, but this activity was reduced after fermentation with LAB.

Conclusion: In conclusion, the findings of this study indicated the isolated LAB showed a typical bacterial growth pattern. While unfermented *Azolla* has moderate antibacterial property, fermentation with LAB reduces its antibacterial effect.

Keywords: *Azolla pinnata*, lactic acid bacteria, antibacterial, solid-state fermentation

*Correspondence: farizan@uitm.edu.my

^a School of Biology, Faculty of Applied Sciences, Universiti Teknologi MARA, Shah Alam, Malaysia