Postbiotics from Indonesian Lactic Acid Bacteria as an Alternative Non-Antibiotic Management for Diabetic Foot Ulcer Infection: An in vitro Study of Antibacterial Activity Against Persister Cells and Biofilms

ITB Research Program (Riset Dosen Muda)

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Abstract

Diabetic foot ulcer infections involve biofilms and antibiotic-tolerant persisters, motivating investigation of postbiotics from Indonesian fermented foods. Within this programme, *Pediococcus acidilactici* On-133 postbiotic (PB133) was evaluated with EDTA against *Staphylococcus aureus*, methicillin-resistant *S. aureus* (MRSA), and *Pseudomonas aeruginosa*. Susceptibility, biofilm, antibiotic potentiation, and keratinocyte compatibility assays were conducted. PB133 at 2.5 mg/mL with 0.625 mM EDTA inhibited biofilm biomass accumulation by 53% to 93%, selectively enhanced antibiotic inhibition zones, and yielded 117% of control HaCaT MTT signals. Established biofilms showed partial responses. These findings support further development of indigenous postbiotics as non-antibiotic adjuvant candidates, with antipersister validation remaining necessary.

Keyword: antibiofilm, postbiotic, diabetic foot ulcer

Introduction

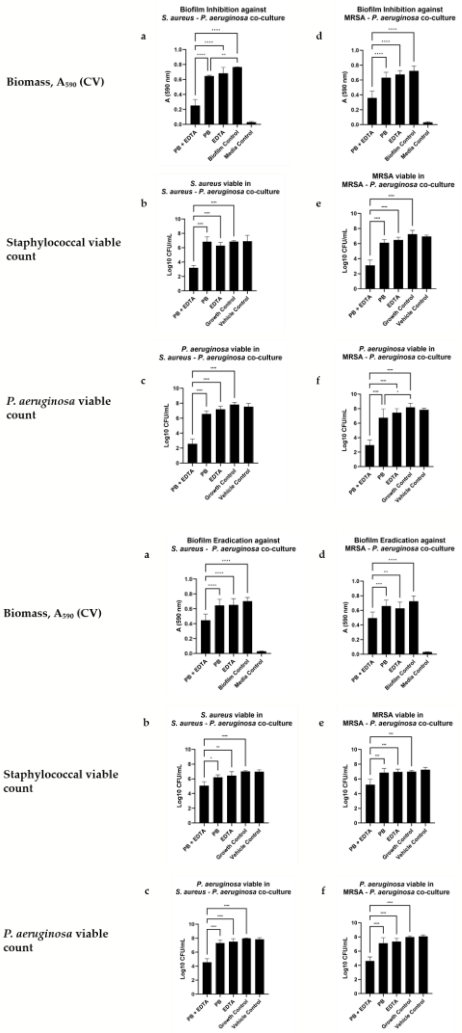
Diabetic foot ulcer infections are complicated by polymicrobial biofilms and antibiotic-tolerant persister cells, which undermine treatment outcomes. Current management relies on debridement and antibiotics, while emerging approaches investigate postbiotics and non-antibiotic adjuvants. However, biofilm research predominantly uses single-species models, and indigenous Indonesian postbiotics remain insufficiently characterised against persister cells and polymicrobial biofilms. This project therefore aims to identify promising postbiotics from Indonesian lactic acid bacteria, evaluate their antibacterial, antipersister, and antibiofilm activities, investigate relevant mechanisms, and assess initial safety. Within this framework, *Pediococcus acidilactici* On-133 postbiotic combined with EDTA is evaluated for biofilm control and selective enhancement of antibiotic activity.

Research Method

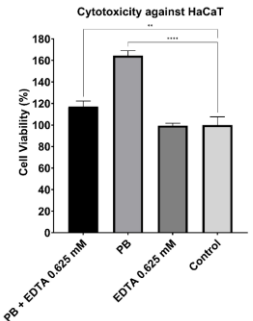
The proposal covers bacterial isolation, molecular identification, postbiotic characterisation, antipersister evaluation, biofilm assays, and toxicity testing. Completed PB133 experiments employed LC-HRMS, microdilution, checkerboard, time-kill assays, crystal violet staining, MTT, viable enumeration, and electron microscopy. Antibiotic enhancement was assessed by monoculture disc diffusion, with HaCaT cells providing an initial cytocompatibility assessment.

Discussion & Result

PB133 and EDTA exhibited synergy in four culture models, with fractional inhibitory concentration indices of 0.250 to 0.375. The combination reduced viable counts in developing dual species biofilms by 3.7 to 5.3 log10 CFU/mL relative to untreated controls. Developing biofilms showed biomass inhibition of 53% to 93%, whereas established biofilms exhibited partial responses. Combination supplementation enlarged antibiotic inhibition zones beyond both individual components and untreated controls for 12 antibiotic-organism pairs. HaCaT MTT signals remained at 117% of control. These results support selective antibiotic enhancement and biofilm suppression, while highlighting the need for improved activity against established biofilms and mechanistic validation.

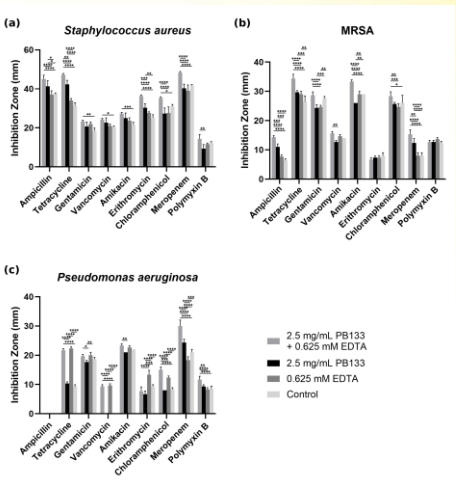


Picture 1. *Biofilm Co-culture Inhibition and Eradication*



Picture 2. *Cytotoxicity against HaCaT of PB133 - EDTA*

PB133 combined with EDTA increased inhibition zones for selected antibiotics, particularly ampicillin and meropenem against MRSA. Responses differed across bacterial species and antibiotic classes, highlighting the importance of combination selection. These findings support further development as a non-antibiotic adjuvant, with potentiation requiring confirmation through broth microdilution and time-kill combination assays.



Picture 3. *Antibiotic potentiation by PB133 - EDTA*

EDTA may facilitate antibiotic access by chelating divalent cations that stabilise bacterial envelopes, while postbiotic metabolites may impose additional physiological stress. However, similar responses to EDTA alone for certain antibiotics suggest that enhancement is not uniformly attributable to PB133. Mechanistic studies are needed to distinguish complementary effects from individual contributions.

Conclusion

This study provides an experimental foundation for developing postbiotics from Indonesian lactic acid bacteria as non-antibiotic adjuvants for infections associated with diabetic wounds. PB133 combined with EDTA demonstrated antibacterial activity, suppressed biofilm formation, and selectively enhanced antibiotic inhibition zones, while maintaining initial compatibility with HaCaT keratinocytes. Responses against established biofilms remained partial, and antibiotic enhancement varied across combinations. Further work should prioritise chemical standardisation, antipersister evaluation, mechanistic confirmation, clinical isolate testing, and representative wound models. Subsequent formulation optimisation, safety assessment, and resistance selection studies are essential to identify candidates suitable for clinical development and substantiate potential contributions to antibiotic stewardship.