Application of Emulsion Biosurfactants from *Bacillus siamensis* KCTC 13613 and Cinnamon Essential Oil as Antibiofilm Agents Against Oral Pathogenic Bacteria

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Abstract

This study characterized the biosurfactant produced by *Bacillus siamensis* KCTC 13613 and evaluated the antibacterial and antibiofilm activities of the biosurfactant, cinnamon essential oil (CEO), and their emulsion against *Streptococcus mutans* and *Enterococcus faecalis*. The biosurfactant was a surfactin-like lipopeptide with a critical micelle concentration (CMC) of 0.1 g/L. It inhibited biofilm formation and eradicated established biofilms from 125 ppm against both species, whereas CEO was effective from 1000 ppm. The emulsion was active from 62.5 ppm biosurfactant combined with 4000 ppm CEO; at 125 ppm biosurfactant + 4000 ppm CEO it eradicated 30.34% (*S. mutans*) and 38.00% (*E. faecalis*) of mature biofilm. All treatments increased extracellular potassium compared with the untreated control, with CEO causing the greatest leakage, suggesting that membrane disruption contributes to the antibacterial activity.

Keywords: biosurfactant, cinnamon essential oil, emulsion, antibiofilm, antibacterial.

Introduction

Indonesia is among the five countries with the highest number of oral disease cases worldwide (WHO, 2022). *S. mutans* is an etiological agent of dental caries, whereas *E. faecalis* is a persistent endodontic pathogen involved in chronic apical periodontitis. Both form biofilms that increase resistance to antimicrobials.

Oral care products commonly contain sodium lauryl sulphate (SLS), which may cause irritation, allergic reactions, and oral mucosal desquamation. Biosurfactants are a more biocompatible, less toxic alternative: they show antibacterial and antibiofilm activity through increased membrane permeability, inhibition of bacterial adhesion, and biofilm eradication.

Cinnamon essential oil (CEO; *Cinnamomum burmannii*) is also antibacterial and antibiofilm, but its hydrophobicity limits solubility and bioavailability. Biosurfactants can act as emulsifiers that improve the dispersion and stability of essential oils in water, potentially enhancing their activity.

Previous research combined biosurfactants from isolates DS-4 and DS-14 with CEO as nanoemulsions against *C. albicans*, *S. mutans*, and *E. faecalis*. This study examines the biosurfactant of *B. siamensis* KCTC 13613: its characteristics, the antibacterial and antibiofilm activity of the biosurfactant, CEO, and their combination, and changes in membrane permeability based on potassium (K^+) leakage.

Acknowledgement

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Research Method

Strain and pathogens. *B. siamensis* KCTC 13613 produced the biosurfactant in SMSSe medium (molasses and urea). Test pathogens: *S. mutans* ATCC 25175 and *E. faecalis* ATCC 29212.

Characterization. FTIR (Shimadzu Prestige 21), LC-MS/MS (TOF MS ES+), and interfacial tension analysis for CMC.

Emulsion. Biosurfactant at 1000, 500, 250, 125, and 62.5 ppm (EM1–EM5), each with 4000 ppm CEO, homogenized by vortexing for 5 min.

MBIC. Glass slides were pretreated with test solutions for 24 h, then incubated 24 h in log-phase cultures; attached cells were counted by plate count. Controls: SLS (positive) and untreated slides (negative).

MBEC. Mature biofilms (32 h *S. mutans*, 36 h *E. faecalis*) on glass slides were exposed to test solutions for 1 min, followed by plate count. Biofilms were visualized with Live/Dead staining and fluorescence microscopy (20×).

K^+ leakage. Cell suspensions were mixed 1:1 with test solutions (biosurfactant, CEO with Tween 80, and emulsion) at MIC-based concentrations and incubated for 5–60 min; K^+ in the supernatant was measured by atomic absorption spectrophotometry (AAS).

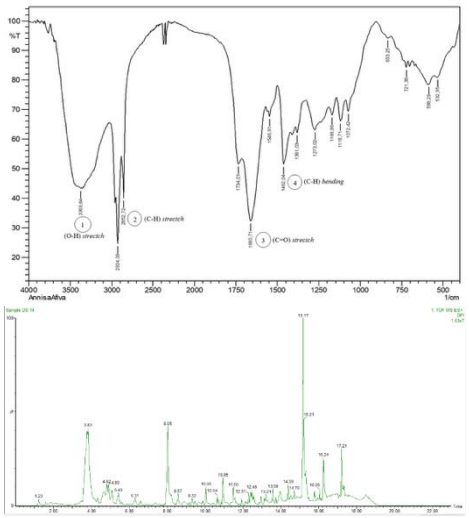


Figure 1. *Characterization of the biosurfactant: FTIR spectrum (top) and LC-MS base peak intensity chromatogram (bottom).*

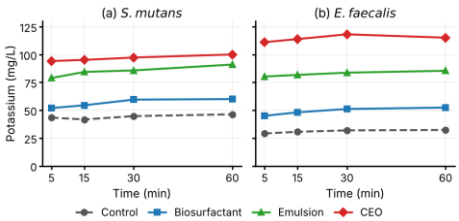


Figure 3. *Extracellular K^+ over 60 min in (a) S. mutans and (b) E. faecalis.*

Discussion & Result

Biosurfactant type. FTIR bands for O–H/N–H (3369.64 cm^{-1}), aliphatic C–H (2924.09, 2852.72 cm^{-1}), ester C=O (1743.01 cm^{-1}), and amide (1660.71 cm^{-1}) indicated a lipopeptide. LC-MS/MS detected C12 surfactin (994.04 m/z, RT 13.568 min); CMC = 0.1 g/L (Figure 1).

Antibiofilm activity (*S. mutans* / *E. faecalis*). Biosurfactant from 125 ppm: inhibition 10.72% / 14.41%, eradication 16.21% / 15.02%. CEO from 1000 ppm: inhibition 11.87% / 11.68%, eradication 22.69% / 19.10%. Emulsion (4000 ppm CEO): inhibition from 62.5 ppm biosurfactant (30.31% / 34.49%); highest eradication at 125 ppm (30.34% / 38.00%). SLS control: 40.18% / 40.32% inhibition; 41.85% / 44.49% eradication (Figure 2).

K^+ leakage. All treatments raised extracellular K^+ above the control within 60 min, in the order CEO > emulsion > biosurfactant (Figure 3).

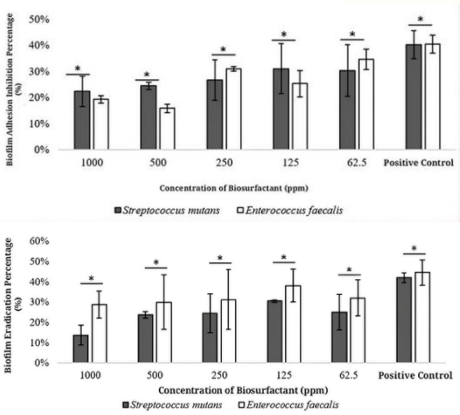


Figure 2. *Biofilm inhibition (top) and eradication (bottom) by emulsions of 4000 ppm CEO with 1000–62.5 ppm biosurfactant (dark bars: S. mutans; white bars: E. faecalis; *, $p \leq 0.05$).*

FTIR and LC-MS/MS data support a surfactin-type lipopeptide. The emulsion inhibited biofilm more than either agent alone at its minimum effective concentration, consistent with complementary actions: the biosurfactant alters surface properties and emulsifies CEO, while CEO (cinnamaldehyde) penetrates the biofilm and disrupts membranes. CEO gave the highest K^+ efflux and the biosurfactant the lowest within 60 min, likely because lipopeptide membrane disruption is time-dependent. The emulsion did not reach the SLS control, so formulation optimization and stability testing are needed.

Conclusion

The biosurfactant of *B. siamensis* KCTC 13613 is a surfactin-like lipopeptide (CMC 0.1 g/L). The biosurfactant (from 125 ppm), CEO (from 1000 ppm), and their emulsion (62.5 ppm biosurfactant + 4000 ppm CEO) showed antibacterial and antibiofilm potential against *S. mutans* and *E. faecalis*. Increased K^+ leakage suggests that membrane disruption contributes.