

The Role of Exopolysaccharides in Matrix Production of *Bacillus subtilis* Biofilms: Resistance to Sanitation in Food Processing

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Structured Abstract

Background: *Bacillus subtilis* is a spore-forming bacterium known for forming robust biofilms in food-processing environments. These biofilms are strengthened by exopolysaccharides (EPS), which serve as structural and protective components within the matrix. EPS contributes to biofilm persistence and resistance against cleaning agents, making sanitation in food facilities highly challenging. This review was conducted to understand the role of EPS in biofilm resistance and to explore effective control strategies.

Methods: This critical review was based on a comprehensive analysis of research articles published between 2014 and 2025. Articles were selected from databases such as ScienceDirect, SpringerLink, and PubMed, with a focus on EPS biosynthesis, biofilm structure, sanitation challenges, and enzyme-antimicrobial treatments. The review synthesised findings on EPS structure, sanitizer penetration, and resistance mechanisms in *B. subtilis* biofilms.

Results: EPS forms a dense matrix that limits sanitizer penetration and chemically neutralizes active agents. Environmental factors like pH, temperature, and metal ions modulate EPS production. Standard sanitizers such as chlorine and PAA are often ineffective due to EPS shielding and induced stress responses that increase EPS synthesis. Enzymes such as glycosyl hydrolases and proteases, especially when combined with antimicrobials like bacteriophages and quorum-sensing inhibitors, showed enhanced efficacy in breaking down the matrix. Surface materials (e.g., stainless steel, rubber) also influence EPS development and biofilm resistance.

Conclusion: EPS plays a critical role in protecting *B. subtilis* biofilms from conventional sanitation methods in food processing. Effective biofilm control requires integrated strategies that combine enzymatic degradation, smart antimicrobial agents, and surface engineering. This review highlights the need for further research into EPS-targeted sanitation approaches and environmental factors that influence biofilm resilience.

Keywords: *Bacillus subtilis*, exopolysaccharides, biofilm resistance, food sanitation, enzyme-antimicrobial combination.

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