Popular Article

TRENDS IN AGRICULTURE SCIENCE

Vol.3 Issue 06 June 2024, Page: 1979-1984

Bacteriocins Fight Against Biofilms

Abarna R*

Dept. of Microbiology, College of Agriculture, Vellayani, Thiruvananthapuram, Kerala 695522

https://doi.org/10.5281/zenodo.12594321

Abstract

Biofilms, consisting of stationary bacterial communities typically enclosed in an extracellular polymeric matrix, shows innate resistance to antimicrobials compared to their free-floating counterparts and are notoriously challenging to eliminate once established. Managing biofilms formed by microbial pathogens is critical for medical researchers because these structures on surfaces of medical devices frequently cause infections in patients. The presence of biofilms shields bacteria from disinfectants and antibiotics, increasing their resistance to eradication efforts. With the scarcity of new antibiotics in recent decades and the rise in resistance among pathogens that form biofilms, there is an urgent need for innovative solutions. One promising approach is the use of bacteriocins as antimicrobials, either alone or combined with existing treatments, to target biofilms. Bacteriocins, which are ribosomally-synthesized antimicrobial peptides (AMPs) ranging from 20 to 60 amino acids long, are characterized by their cationic and hydrophobic properties and can inhibit both gram-positive and gram-negative bacteria, presenting a fresh strategy for combatting them. Some bacteriocins, such as nisin, have demonstrated bactericidal and anti-biofilm effects against a variety of bacterial species, highlighting their potential clinical significance. These antimicrobial peptides act through diverse mechanisms, including pore formation and inhibition of cell wall, nucleic acid, or protein synthesis. However, the growing resistance of food spoilage and pathogenic bacteria to bacteriocins is becoming concerning, prompting research efforts to understand and address this resistance issue.

Keywords: Bacteriocins, Biofilm, nisin, antibiotics

Introduction

Bacteria typically exist in one of two primary forms of population: planktonic, where they freely reside in a liquid medium, and sessile, where they are attached either to a surface or within a biofilm structure. Bill Costerton in 1978, coined the term "biofilm" which denotes complex assemblies composed of diverse microorganism communities enclosed within a matrix, predominantly comprising exopolysaccharides. This matrix enables their adhesion to inert or organic surfaces, such as rocks, glass, plastic, skin, cuticle, or mucosa. Biofilms consist of cells immobilized on a substrate and often embedded within an organic polymer matrix of microbial origin. These structures are biologically active, composed of cells and extracellular substances, firmly adhering to solid surfaces. Biofilms represent sessile microbial communities thriving on surfaces, typically embedded in an extracellular polymeric substance matrix. Within biofilms, cells aggregate into clusters ensconced within a self-produced extracellular matrix rich in

Published: 28 June 2024
ISSN: 2583-7850

biomolecules like polysaccharides, proteins, nucleic acids, and lipids. This mode of growth predominates among bacteria across natural, industrial, and clinical environments, where densely packed multispecies populations of cells reside within a self-synthesized polymeric matrix, firmly attached to tissues or surfaces.

Biofilm development:

Biofilm formation represents a widespread bacterial characteristic with distinct developmental stages. These stages include initial reversible attachment, followed by irreversible attachment, maturation involving the growth of a monolayer and production of extracellular polymeric substances (EPS), and dispersion of mature biofilm cells into the environment (Figure 1). Initially, planktonic bacteria make initial contact with a surface, a reversible phase where they subsequently form a monolayer and secrete EPS for protection (Berlanga and Guerrero, 2016). The matrix consists of extracellular polysaccharides, structural proteins, cell debris, and nucleic acids, primarily extracellular DNA (eDNA) initially, transitioning to polysaccharides and proteins. Microcolonies form during these stages, facilitated by cell-cell communication mechanisms like quorum sensing. Biofilm growth becomes three-dimensional with irreversible attachment, culminating in the dispersion of mature biofilm cells back into planktonic form, potentially initiating another cycle of biofilm formation.

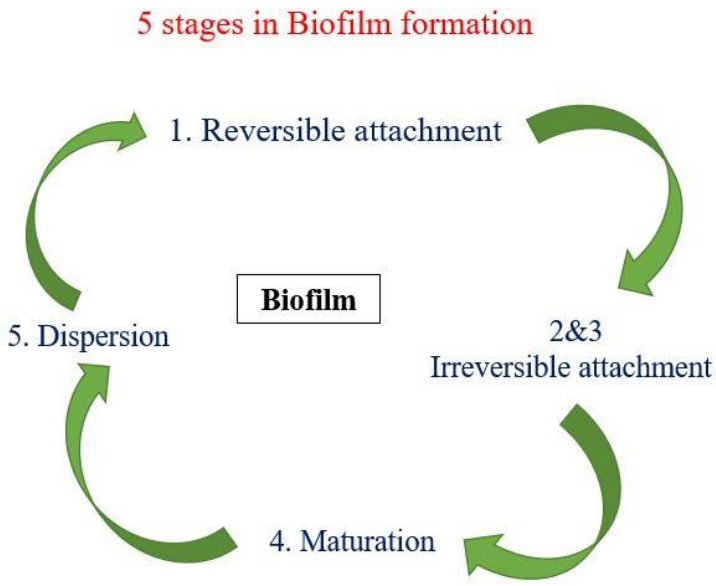

Figure 1: Biofilm formation

Quorum sensing

Biofilm development and quorum sensing (QS) are intricately linked processes in bacterial communities. Biofilms represent a cooperative behavior among bacterial populations residing within a self-produced extracellular matrix. QS serves as a cellular communication mechanism, coordinating gene expression based on population density through specific signaling molecules. These molecules, such as acyl homoserine lactones (AHLs) and peptides, accumulate

Published: 28 June 2024
ISSN: 2583-7850

until a threshold population density trigger coordinated responses. The question arises as to when QS signaling impacts biofilm regulation, suggesting that initial adhesion stages, involving freely swimming bacteria, are unlikely sites for quorum signal accumulation. Instead, as attached bacteria proliferate into microcolonies, population density rises, enabling QS signals to regulate biofilm maturation and disassembly. Recent studies indicate QS involvement in biofilm dispersal, crucial for bacterial migration to new environments amidst resource limitations. QS networks, complex and gene-rich, govern biofilm dynamics, influencing stages from synthesis to dispersion, though their precise mechanisms remain intricate and multifaceted.

Problems with the biofilms

Bacterial cells within biofilms display increased resistance to both the host's immune responses and antibiotic therapies. Consequently, once biofilms adhere to surfaces of medical devices like vascular catheters, prosthetic joints, and pacemakers, they become exceedingly challenging to eliminate, often necessitating invasive procedures such as device removal. These biofilm-related infections tend to be persistent or recurring, underscoring the critical need for effective prevention and treatment strategies. Biofilms are linked to various chronic diseases such as tooth decay, periodontitis, and otitis media, as well as healthcare-associated infections like pneumonia and urinary catheter cystitis. They contribute significantly to antimicrobial resistance, rendering conventional antibiotic treatments largely ineffective. In the food industry, biofilms pose a risk of causing outbreaks of foodborne diseases. Moreover, inadequate cleaning practices in hospitals may exacerbate the spread of resistance. Efforts have therefore focused increasingly on preventing initial biofilm formation rather than targeting fully developed biofilms. Strategies targeting biofilm adhesion, quorum sensing, and dispersion have been explored as crucial steps in biofilm formation. Dental plaque, the most prevalent biofilm in humans, notably in the oral cavity, plays a central role in causing dental caries and periodontal diseases. Researchers have investigated various approaches to mitigate the adverse effects of dental plaque biofilms, particularly targeting *Streptococcus mutans*, a key organism implicated in dental biofilm formation and caries development.

Bacteriocins

The identification and development of antibiotic therapy can be considered one of the most impactful scientific achievements of the twentieth century in terms of reducing human morbidity and mortality. However, challenges have emerged that hinder their effectiveness in the twenty-first century. Pathogens have developed resistance to both single and multiple antibiotics, compounded by a scarcity of new antibiotic classes due to the high costs and risks involved in their development. Consequently, alternative therapeutic approaches beyond traditional antimicrobial therapies are urgently needed. Bacteriocins have emerged as promising candidates,

Published: 28 June 2024
ISSN: 2583-7850

being bacterially produced peptides or proteins typically 2–10 kDa in size. These molecules exhibit bacteriostatic and/or bactericidal effects against bacteria, showing potential as substitutes for or supplements to current antimicrobial agents. Bacteriocins possess advantageous properties such as potent activity, stability, low toxicity, and a wide spectrum of activity, making them suitable for clinical use.

Classification

Bacteriocins synthesized by Gram-positive bacteria fall into two main categories: class I comprises lantibiotics, distinguished by their incorporation of unusual amino acids like lanthionine and dehydroalanine through posttranslational modifications, resulting in cyclic structures that enhance stability under various environmental stresses. In contrast, class II bacteriocins are small peptides that lack such modifications, classified by Cotter *et al*., (2005) into groups IIa (pediocin-like), IIb (two-peptide), IIc (cyclic), and IId (linear). Notably, nisin A, a type AI lantibiotic from Lactococcus lactis, acts both bacteriostatically by blocking lipid II, a cell wall precursor, and bactericidally by forming pores upon membrane insertion (Kumariya *et al*., 2019). Conversely, nukacin ISK-1, a type AII lantibiotic from Staphylococcus warneri ISK-1, exhibits only a bacteriostatic mode, inhibiting cell wall synthesis without pore formation. Lacticin Q, a class IId bacteriocin from Lactococcus lactis QU 5, operates bactericidally by forming large toroidal pores that induce protein leakage and lipid flip-flop in target cells, not reliant on specific receptors like lipid II for its action.

Mechanism of Action

Bacteriocins exert bactericidal effects through distinct mechanisms targeting either the cell envelope or intracellular processes, impacting gene expression and protein synthesis. For instance, Nisin and certain lantibiotics inhibit cell wall biosynthesis by binding to lipid II, crucial for transporting peptidoglycan monomers across the cytoplasmic membrane, a site also targeted by vancomycin. This differential binding allows bacteriocins to maintain activity against vancomycin-resistant gram-positive pathogens. Most bacteriocins induce cell membrane permeabilization, leveraging specific receptors on target cell surfaces for their action. Nisin and lantibiotics utilize lipid II to facilitate pore formation, leading to membrane potential loss and cell death. Additionally, various bacteriocins, such as pediocin-like bacteriocins, lactococcins A and B, and MccE492, target the mannose phosphotransferase system (Man-PTS) on sensitive cells, inducing pore formation. Recently identified receptors include the maltose ABC transporter for class IIc bacteriocins, garvicin ML's Zn-dependent metallopeptidase for class IId bacteriocin LsbB, and an undecaprenyl pyrophosphate phosphatase for class IIb bacteriocin lactococcin G.

Resistance development in bacteria against bacteriocins:

Published: 28 June 2024
ISSN: 2583-7850

Organisms inherently possess the capacity to adapt to changing environments. Consequently, target bacteria also develop defensive mechanisms against bacteriocins upon prolonged exposure, resulting in bacteriocin resistance. Bacteriocins have been employed for decades, yet resistance remains a longstanding issue. Resistance in these bacteria arises through various mechanisms. Firstly, alterations occur in membrane receptors which act as binding sites for bacteriocins. Depending on their type, bacteriocins bind to specific targets such as lipid II, permease mannose phosphotransferase system, undecaprenyl pyrophosphate phosphatase (UppP), maltose ABC transporter, permease, or zinc-dependent membrane-bound proteases. Reduced expression of the mannose-specific phosphotransferase system (Man-PTS) gene in bacteriocin-resistant *Listeria monocytogenes* suggests a lower density of receptors, thereby impairing bacteriocin binding. Secondly, resistance can stem from modifications to teichoic acids of the cell wall, diminishing its negative charge and altering its composition. Teichoic acids, phosphate-rich polymers anchored to the membrane via glycolipids, typically impart a negative charge to the cell wall. However, D-alanylation transforms teichoic acids into positively charged entities, neutralizing their anionic properties. Additionally, some bacteria counteract bacteriocins by L-lysinylation of anionic phospholipids, forming lysylphosphatidylglycerol, a basic phospholipid that enhances membrane positivity and shields against bacteriocins like daptomycin. Lastly, changes in the composition of cell membrane fatty acids, increasing saturated and branched-chain fatty acids, enhance membrane rigidity and reduce fluidity, thereby impeding bacteriocin penetration. Interestingly, this adaptation mirrors the strategies pathogens employ to evade antibiotics such as ampicillin, chloramphenicol, erythromycin, and tetracycline, highlighting bacteriocins as a subset of antibiotics.

Approaches to mitigate the bacteriocin resistance:

Bacteriocin alone may not suffice to control food-spoiling bacteria. Excessive dosages of bacteriocin could lead to non-specific bactericidal effects, posing potential hazards to consumers. Therefore, combining bacteriocin with other antimicrobial agents is recommended. Plant essential oils, such as thyme, cumin, rosemary, basil, coriander, mint, sage, lavender, oregano, and cinnamon derivatives, have shown promise as food preservatives. These oils contain phytochemicals like terpenes, phenolics, cinnamaldehyde, p-cymene, and allyl isothiocyanate. For instance, oregano essential oil (0.6%) combined with nisin (500 IU/g) effectively inhibited *Salmonella enteritidis* in minced meat. Similarly, thyme essential oil (0.6%) paired with nisin (500–1000 IU/g) suppressed the growth of *L. monocytogenes* (Mathur *et al*., 2018). Allyl isothiocyanate, in combination with nisin, demonstrated inhibitory effects against *L. monocytogenes* and *S. aureus*. Additionally, irradiation has been shown to enhance the efficacy

Published: 28 June 2024
ISSN: 2583-7850

of bacteriocins. Encapsulated nisin, along with oregano and cinnamon essential oils, increased the susceptibility of *L. monocytogenes* to γ-irradiation.

In addition to pairing bacteriocins with other antimicrobial compounds, combining two bacteriocins has also shown increased potency against their targets (Rocha *et al*., 2019). Notably, the combined use of bacteriocins significantly reduces the minimum inhibitory concentration required, thereby lowering inhibitory dosages. Numerous studies have highlighted the advantages of using paired bacteriocins, such as the combinations of nisin with pediocin AcH, nisin with leucocin F10, and lacticin 481 with pediocin AcH. Both nisin and curvaticin 13 have exhibited increased effectiveness in combating *Listeria monocytogenes* when administered together or in a sequential manner. Recently, the combination of bacteriocin AS-48 and nisin demonstrated enhanced efficacy against Staphylococci compared to individual bacteriocins, suggesting that this strategy provides an alternative approach to combat bacteriocin resistance. An additional developing approach includes the methodical development of bacteriocins to augment their effectiveness and operational capabilities.

Conclusions

In conclusion, while bacteriocins offer a promising alternative to synthetic antimicrobials, their effective application requires addressing challenges such as susceptibility to diverse bacterial species and the potential for resistance development. Strategies involving isolation, characterization, and engineering of bacteriocins are crucial for enhancing their stability and efficacy. Combining bacteriocins with different mechanisms of action or with synthetic antimicrobials may mitigate resistance and broaden their utility. However, careful consideration of their potential hazards and exploration of alternative preservation methods are essential to harnessing bacteriocins' full potential in food safety and therapeutic applications while minimizing risks associated with resistance development and toxicity.

References

Berlanga, M. and Guerrero, R., 2016. Living together in biofilms: the microbial cell factory and its biotechnological implications. *Microbial cell factories*, *15*, pp.1-11.

Cotter, P.D., Hill, C. and Ross, R.P., 2005. Bacteriocins: developing innate immunity for food. *Nature Reviews Microbiology*, *3*(10), pp.777-788.

Kumariya, R., Garsa, A.K., Rajput, Y.S., Sood, S.K., Akhtar, N. and Patel, S., 2019. Bacteriocins: Classification, synthesis, mechanism of action and resistance development in food spoilage causing bacteria. *Microbial pathogenesis*, *128*, pp.171-177.

Mathur, H., Field, D., Rea, M.C., Cotter, P.D., Hill, C. and Ross, R.P., 2018. Fighting biofilms with lantibiotics and other groups of bacteriocins. *npj Biofilms and Microbiomes*, *4*(1), p.9.

Rocha, K.R., Perini, H.F., de Souza, C.M., Schueler, J., Tosoni, N.F., Furlaneto, M.C. and Furlaneto-Maia, L., 2019. Inhibitory effect of bacteriocins from enterococci on developing and preformed biofilms of *Listeria monocytogenes*, *Listeria ivanovii* and *Listeria innocua*. *World Journal of Microbiology and Biotechnology*, *35*, pp.1-11.

Published: 28 June 2024
ISSN: 2583-7850