Kinetic microplate assay reveals lethal and sub-lethal behavior of antimicrobials immobilized on solid substrates

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Statement of the Problem: Immobilization of antimicrobials onto surfaces is of great interest, although characterization of activity can be problematic. Traditional assays are designed for determining solution based antimicrobial activity and is incompatible with solid substrates.

Methodology & Theoretical Orientation: A kinetic microplate method was developed to determine the minimum bactericidal concentration (MBC) of the immobilized antimicrobial peptides SMAP and Cecropin P1 through a combination and modification of traditional solution assays, overcoming the difficulties of working with a solid substrate. The microplate-based kinetic assay was used to measure various peptide dose and time-dependent activity at multiple concentrations; viable plate counts were used to determine bactericidal activity and correlated to the kinetic assay results.

Findings: Immobilized peptide activity against both Gram-positive and Gram-negative bacteria has been demonstrated, including *Acinetobacter baumannii*, *Bacillus anthracis sterne* and *Staphylococcus aureus*, and correlated to viable plate count results. Compared to peptides in solution, a combination of increased concentration and longer exposure time was required for activity. Immobilized peptide potency was cell–dependent; however, the peptides exhibited activity for all organisms in a dose-dependent manner, reaching a critical concentration that resulted in complete inhibition. The role of immobilized peptide orientation relative to the solid substrate revealed that orientation is critical to activity.

Conclusion & Significance: This assay successfully determined activity on magnetic beads and planar glass substrates; other substrates such as antimicrobial textiles could also be characterized with this technique. Furthermore, the method yields information regarding sub-lethal concentrations not realized in the traditional assays. This kinetic microplate assay is also useful for testing antimicrobial formulations and reveals both synergistic and antagonistic interactions against clinical isolates and biothreat agents..

Speaker Biography

Steven Arcidiacono has an MS in Microbiology, with significant contributions in the research areas of anaerobic fermentation, antimicrobials, and biopolymer fermentation/fiber spinning. His primary focuses encompass exploratory research and development studies in the following specific areas: 1) colonic fermentation for biotransformation of nutritional and polyphenolic compounds; 2) skin microorganism interaction when in co-culture; and 3) discovery of novel antimicrobials to combat multidrug resistant bacteria and fungi. His prior experience/programs include antimicrobial peptides for detection and microbial protection and aqueous spinning of biopolymers (naturally-derived crystallin proteins and recombinant spider silk). He is Author/Coauthor of >30 peer-reviewed manuscripts, book chapters, proceedings and conference articles (cited >1500 times), numerous presentations, and an inventor on two issued US patents and two patent applications.

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