Antibiofilm effect of Temporin-L on Pseudomonas fluorescens, in static and dynamic conditions.

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Introduction

Biofilm consists of a complex self-produced matrix of polysaccharides, DNA and proteins that protects bacteria from the environment including the host immune system and constitutes the main cause of bacterial resistance against antibiotics. Research is then focused on finding alternative antimicrobial substances able to either hamper biofilm formation or to prevent bacterial growth. Recently, we showed that the antimicrobial peptide Temporin-L impairs E.coli growth by inhibiting cell division (Di Somma et al.; 2020; BBA). Here we investigate the effect of Temporin-L (TL) on biofilm formation in Pseudomonas fluorescens (P. fluorescens) both in static and dynamic conditions, showing that TL displays antibiofilm properties.

Materials and methods

Biofilm formation in static conditions was performed on coverslips and analyzed by the Crystal Violet assay. Biofilm morphology was assessed using imaging techniques. Investigation of biofilms in dynamic conditions was performed in a flow chamber using a microfluidic system and images were recorded by confocal microscopy.

Results

The P. fluorescens cells were either grown in the presence of TL or incubated with the antimicrobial peptide after biofilm formation both in static and dynamic conditions using different concentrations of the peptide. When TL was added during cell growth, the peptide affected biofilm formation at 25 µM. Confocal microscopy demonstrated that at this concentration P. fluorescens cells were still alive but a clear disruption of the biofilm architecture was observed. These results had to be ascribed to
a specific antibiofilm effect of TL. At 100 µM TL antibiofilm activity biofilm thickness was nearly negligible.

When P. fluorescens cells were treated with TL following biofilm formation, confocal images demonstrated that the peptide exerted a strong antibiofilm effect leading to cell detachment and disruption the biofilm architecture.

Discussion and Conclusions

Investigation of TL effect on P. fluorescens showed that when added during bacterial growth this peptide exerted antibiofilm activity at low concentration impairing biofilm formation both in static and dynamic conditions, leaving most of bacterial cells still alive. However, confocal microscopy measurements could not detect the long necklace-like structures observed in E.coli indicating a different mechanism of action of TL on P. fluorescens. Furthermore, when TL was added to a preformed P. fluorescens biofilm, the peptide showed a strong antibiofilm activity both in static and dynamic conditions, suggesting that TL might penetrate biofilm architecture with a still unknown mechanism leading to disruption of P. fluorescens biofilm.