

Blocking Intra-Bacterial Communication to Improve Water Quality in Developing Countries

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Microbial biofilm formation in pipes delivering drinking water contributes to nearly 80% of water-borne illnesses in developing countries [1]. *Pseudomonas fluorescens* is one potential biofilm-forming bacteria contributing to this problem [2]. *P. fluorescens* is known to coordinate biofilm formation by secreting communication molecules [3]. This study presents novel data suggesting that *P. fluorescens* strain B-253 produces N-Acyl homoserine lactones (AHLs), representing at least one of their communicating molecules. Peptide LL-37 decreases biofilm formation, *ex vivo*, through a mechanism that has yet to be fully elucidated [4]. Current research focuses on preventing biofilm growth in clinical hospital settings, however, the novel approach reported here focuses on preventing biofilm formation in the water supply to decrease the number of infections caused by contaminated pipelines.

The objective of this study was to identify the effect of Peptide LL-37 on the production of AHLs by *P. fluorescens*, which would have implications in defining the mechanism by which LL-37 has been found to modulate biofilm formation *ex vivo*. The quorum sensing mechanism of *P. fluorescens* B-253 was specifically observed when introduced to LL-37. *P. fluorescens* were treated with varying concentrations of LL-37 (0.01 µg/mL – 10 µg/mL) to determine if AHL production was altered. Using an AHL reporter strain of *Rhizobium radiobacter*, data suggested that LL-37 modulated AHL production in *P. fluorescens* through a biphasic, low-dose inhibitory effect with higher doses causing an increase in production of the signaling molecule ($p < 0.05$). This suggests that there was potentially an optimal low dose response to the peptide in decreasing AHL production. Perhaps, higher concentrations of peptide lead to increased stress on the bacteria, which increase bacterial communication, correlating with the production of AHLs. This warrants further investigation by measuring stress response molecules, such as molecular chaperones.

The effect of LL-37 on bacterial viability was observed, previously unreported in other studies in the context of AHL production and biofilm formation by this organism. LL-37 treatment caused a low-dose proliferative effect on bacterial viability, with higher doses of the peptide reducing colony forming units (CFUs) ($p < 0.05$), representing an inverse relationship to AHL production. It is possible that at high concentrations of LL-37, viability decreased due to the increasing toxicity of LL-37, or, perhaps increased AHL concentration accompanies reduced viability as the organisms expend energy synthesizing the communication molecules, as opposed to proliferation. Most interestingly, perhaps is the possibility that AHL communication involves transcription of genes involved in the modulation of bacterial viability. Data also demonstrated decreased biofilm production, most pronounced at low-doses of LL-37 ($p < 0.01$). LL-37 may modulate biofilm formation in direct relationship to AHL modulation, suggesting a potential mechanistic relationship, whereas LL-37 decreases biofilm formation via the production of AHLs in *P. fluorescens*.

This research holds potential in the future to improve the quality of potable water that is being contaminated by these species of microbes in pipelines of developing countries.

References:

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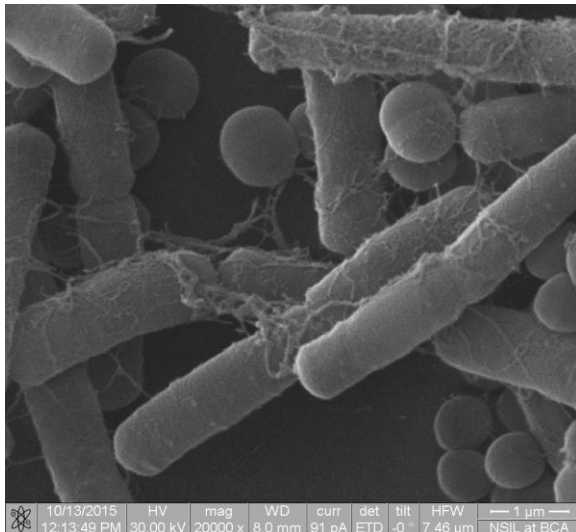


Figure 1. SEM imaging of biofilm formation after 24h. Rod-like structures are *P. fluorescens*; string-like substance between the bacteria are EPSs secreted by the bacteria to form biofilms.

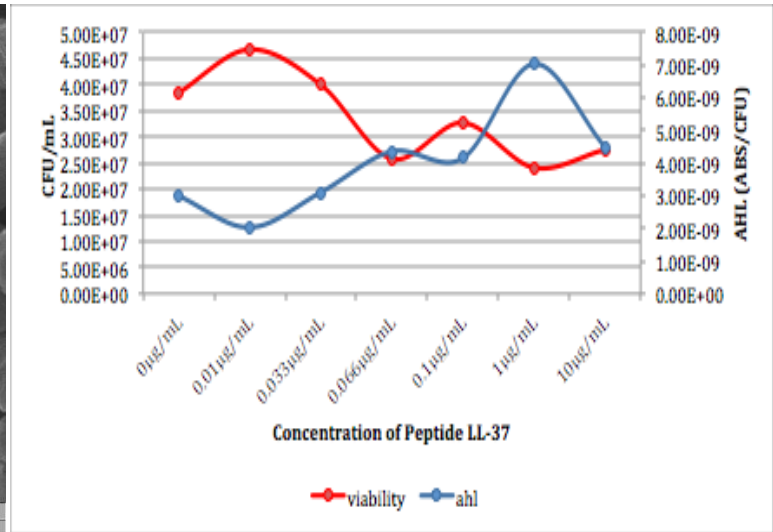


Figure 2. Effect of LL-37 on Bacterial Viability and AHL per CFU. Viability is inversely proportional to production of AHLs per CFU. With increasing concentrations of LL-37, a decrease in viability and an increase of AHLs is shown. Samples read at 630 nm.

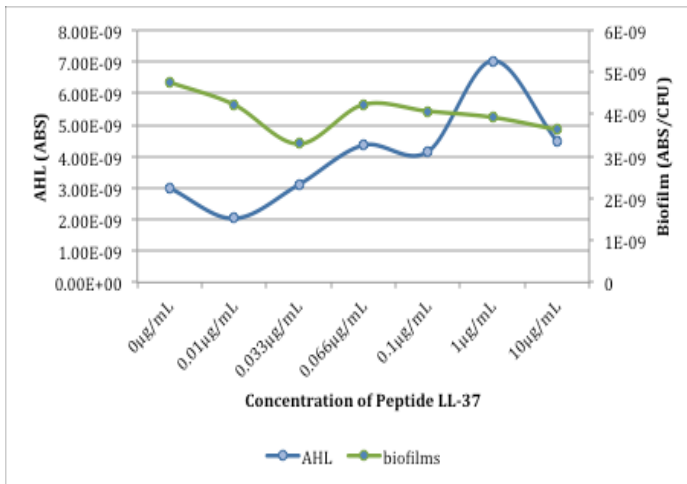


Figure 3. Effect of LL-37 on Biofilm Formation and AHLs. Data suggests a direct relationship between biofilm production and AHLs. Samples read at 570 nm for biofilm formation and 630 nm for AHLs.

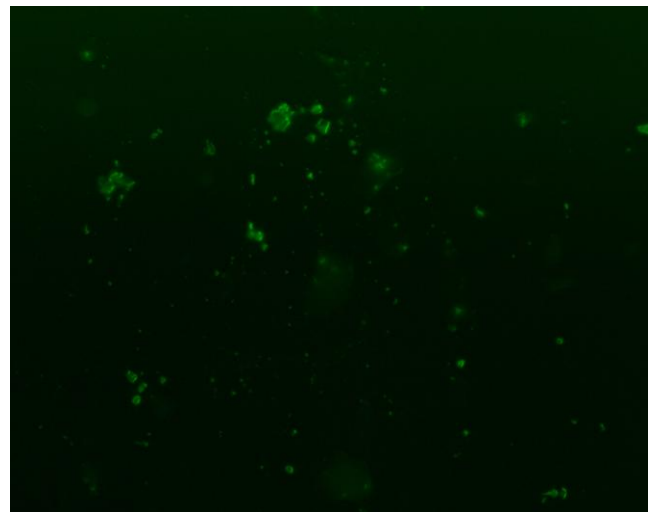


Figure 4. Fluorescence imaging of bacteria were taken after 24h of being introduced to LL-37. Fluorescence was used as another measure of viability of the bacteria. Image was acquired at 200X magnification.