The mechanism of cationic antimicrobial action in *Bacillus subtillis*.

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Introduction

The mechanism of bacteria lysis by positively charged(cationic) antimicrobial agents is reputed to be due to the electrostatic attraction between the bacterial cell wall and the antimicrobial agents. The *dlt* operon in *bacillus subtillis* is responsible for the addition of D -alanine to the negatively charged teichoic acids on its cell wall. This D-alanylation is important during lysozyme resistance. This is because D-alanine which is positively charged is added to the cell wall making it less negative and causing electrostatic repulsion between the positively charged lysozyme and the less negative cell wall. Less lysozyme therefore attach to the cell wall and hence resistance is achieved. Here, we show that there is more to the mechanism than electrostatic interaction alone.

Aim: To demonstrate the mechanism of cationic anti-microbial lysis by using *B.subttllis* lacking the *dlt* operon(*Δ dlt*).

Method

- The *Δdlt* mutant and the wild type strains(168) were cultured overnight at 30°C using PAB medium. The cultures were then incubated at 37°C to log phase. They were then treated with either lysozyme, LL-37 autoimmune peptide, polylysine or fluorescently labelled ploysine(FITC-Polylysine).
- Samples were taken from the treated cultures and plated onto agar plates at time t0 and then at 5 minutes intervals. Microscopy was done using samples containing the FITC-polylysine.

Results.

- The *Δdlt* was more sensitive to the human autoimmune peptide(LL-37) than the wild type (see graph 2)
- The *Δdlt* was also more sensitive to lysozyme(See graph 1)
- There was no significant difference in sensitivity between the two strains to polylysine.(see Figures 1 and 2).

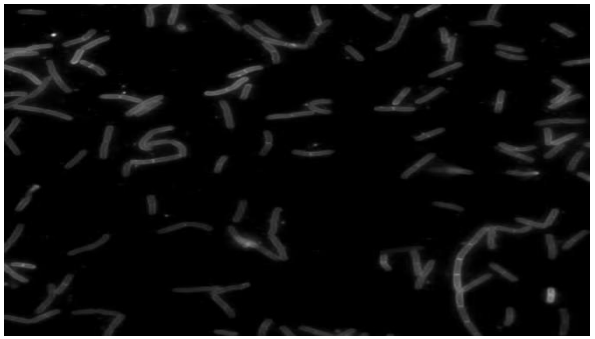


Figure1: FITC-polylysine treated DLT strain.

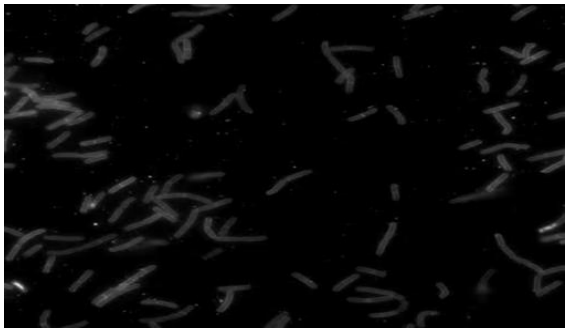
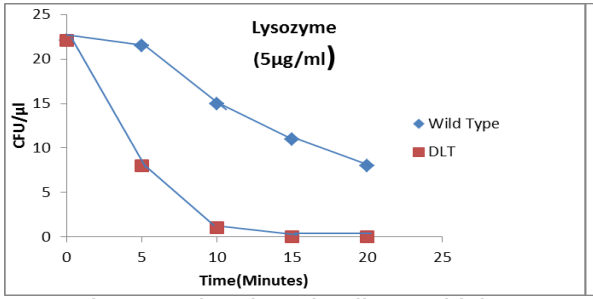
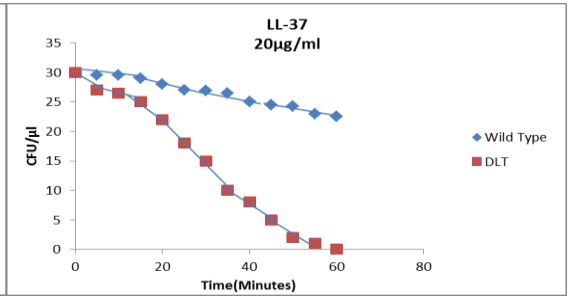


Figure 2: FITC-polylysine treated 168 strain



Graph 1: WT(168) and *Δdlt* sensitivity to lysozyme.



Graph 2. WT(168) and *Δdlt* sensitivity to LL-37.

Discussion and Conclusions:

The higher sensitivity of the *Δdlt* mutant to the cationic antimicrobial agents(CAA) is congruent with the studies done by Khattar et al and others. However, these studies have focussed on the increased electrostatic attraction due to the inability of the *Δdlt* mutant to make D-alanine at the expense of the action of these agents on the cell wall. We have shown here that an equally important aspect of the lysis mechanism is the action of the CAAs on the wall itself. This conclusion was drawn from the polylysine control, a positively charged molecule that could only induce lysis by accumulating on the surface of the bacteria. The *Δdlt* mutant was not more sensitive to polylysine. Therefore, more polylysine molecules on the surface of *Δdlt* mutants, did not translate to increased sensitivity. Time did not permit us to investigate this mechanism but this will be a very worthwhile area of research.

References

- Z. Abi Khattar, A. Rejasse D. Destoumieux-Garzón 2009. The dlt Operon of Bacillus cereus Is Required for Resistance to Cationic Antimicrobial Peptides and for Virulence in Insects Bacteriol. 7063.