Characterising the biofilm forming capabilities of Cystic Fibrosis *Pseudomonas aeruginosa* clinical isolates

G. Whitaker[1], V. Urbach[2,3], B. Dimitrov[4] H. Humphreys[1,5], **N. T. Stevens[1]**
Departments of [1]Clinical Microbiology & [2]Molecular Medicine, RCSI, Dublin 9. Ireland
[3]National Children's Research Centre, Our Lady's Children's Hospital, Crumlin, Dublin 12. Ireland [4] Academic Unit of Primary Care & Population Sciences, University of Southampton, Southampton General Hospital, Southampton SO166YD, England, UK [5]Department of Microbiology Beaumont Hospital, Dublin 9. Ireland

Abstract

Cystic fibrosis (CF) is caused by mutations in the cystic fibrosis conductance regulator (*CFTR*) gene. In the lungs, CFTR dysfunction results in dehydration of the airway surface liquid and reduced mucus clearance. This supports colinisation of the airway with *Pseudomonas aeruginosa* and production of a biofilm. Biofilm formation is influenced by local environmental conditions such as oxygen availability, osmolarity and pH. The biofilm forming capabilities of eight *P. aeruginosa* CF isolates and the biofilm-positive strain PAK were investigated under varying *in vitro* growth conditions using a microtitre plate biofilm assay. Pulsed-field gel electrophoresis confirmed there was clonal variability amongst the clinical isolates. Under aerobic growth conditions, 6 (75%) of the isolates and PAK were strongly adherent in Tryptic Soya Broth (TSB) supplemented with 0.5% NaCl. The effect of pH on biofilm production was investigated by varying the concentration of glucose in the growth media. Using PAK, it was found that there was a significant increase in biofilm formation in TSB supplemented with 1% and 2% glucose compared to TSB alone ($P \leq 0.022$ & $P \leq 0.019$ respectively). This was associated with a small but significant decrease in pH of the growth media from pH 8.45 in TSB alone, to pH 7.83 in TSB + 1% glucose and pH 6.77 in TSB + 2% glucose ($P \leq 0.022$ respectively). Five (62.5%) of the clinical isolates were strongly adherent when the media was supplemented with 1% glucose. Anaerobically, isolates were either weakly adherent or non adherent in all conditions. Biofilm formation was variable but most isolates became more adherent with the addition of NaCl or glucose alone. The effect of pH on biofilm appears to variable in these isolates. Understanding the factors influencing the pathogenesis of *P. aeruginosa* biofilm infections in the CF lung is necessary for better management of these patients.

Background & Aims

P. aeruginosa is a Gram-negative bacillus that causes chronic lung infections in CF patients. Continuous activation of innate immune responses in the lung results in progressive destruction of the lung .Treatment is complicated due to *P. aeruginosa* alginate production and biofilm formation. This is because biofilm cells have an innate resistance to antibiotics. Biofilm formation is influenced by local environmental conditions such as oxygen availability, osmolarity and pH. Mature biofilm formation is characteristic of chronic infection in the airway of CF individuals. Understanding the influence of such factors on biofilm development is necessary if we are to find new therapeutic approaches to treating biofilm-related infections in CF patients.

Aims:

- To investigate the biofilm forming capabilities of *P. aeruginosa* CF clinical isolates under varying *in vitro* environmental conditions
- To investigate the effect of pH on *P. aeruginosa* *in vitro* biofilm development.
- To determine the existence of clonal relationships between the clinical isolates

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Methods

Bacterial strains and isolates: Clinical isolates were collected from sputum specimens obtained from CF patients attending Beaumont Hospital. Control strain, PAK, was obtained from Prof. Alain Filloux, Imperial College, London, UK.

Microtitre Plate Biofilm assay: Biofilm assays were performed in Tryptic soya broth (TSB) alone or TSB supplemented with NaCl (0.5-2%) and glucose (0.5-2%) under aerobic and anaerobic growth conditions for 48 h. Absorbance of adhered stained cells was obtained at 595 nm and biofilm phenotypes were defined according to the criteria outlined by Stepanovic *et al.*[1]

Pulsed field gel electrophoresis: clonal variability amongst clinical isolates was investigated using PFGE. Following staining with Ethidium bromide. Banding patterns were analysed using GelCompar II® software.

Results

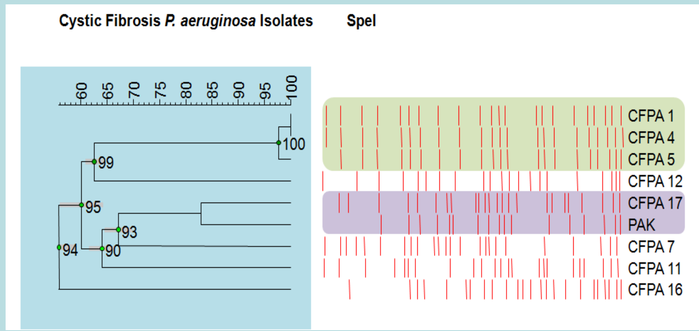

Figure 1. The dendrogram was constructed with GelCompar II ® software (Ver 6.5) by clustering analysis determined using the Dice coefficient (tolerance 1%). Clonal groups were based on a similarity of ≥80% (as indicated by the highlighted groups).

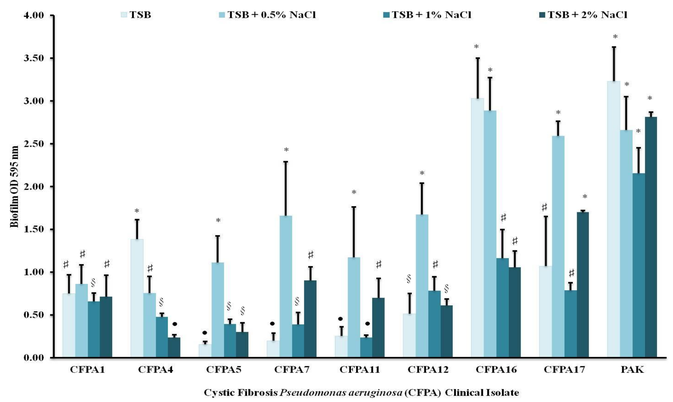

Figure 2. Effect of NaCl on biofilm forming capabilities of CF *P. aeruginosa* isolates under aerobic growth conditions. Graphs show mean Biofilm OD at 595 nm. Biofilm conditions were Tryptic soy broth (TSB), TSB + 0.5%, 1%, and 2% NaCl,. Isolates were identified as non-adherent (•), weakly adherent (§), moderately adherent (#) or strongly adherent (*).

➢Anaerobically isolates were non-adherent or weakly adherent under all growth conditions(data not shown)

Results

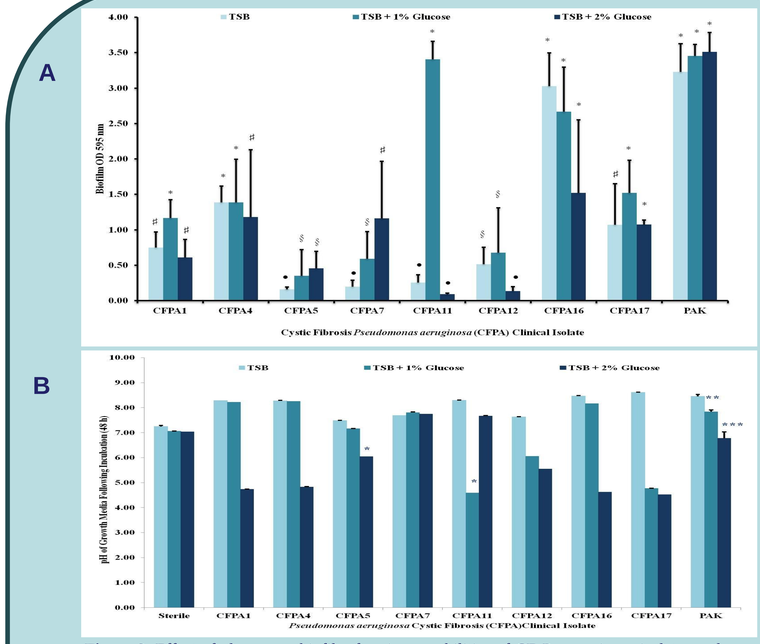

Figure 3. Effect of glucose on biofilm forming capabilities of CF *P. aeruginosa* isolates under aerobic growth conditions (A) and on pH of growth media following aerobic biofilm growth (B) Graphs show mean Biofilm OD at 595 nm. Biofilm conditions were TSB, TSB + 1% glucose and TSB + 2% glucose. Isolates were identified as non-adherent (•), weakly adherent (§), moderately adherent (#) or strongly adherent (*). There was a significant increase biofilm formation in CFPA5 and CFPA11 with the addition of 2% and 1% glucose respectively ($P \leq 0.0001$). There were significant decreases in pH of the media in which these biofilms formed (*) where $P \leq 0.0001$. For PAK (** and ***) denotes a significant decrease in pH compared to TSB alone where $P \leq 0.022$.

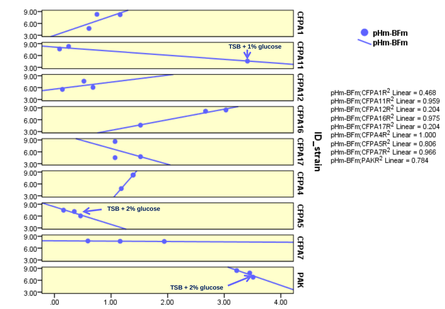

Figure 4. Correlation between biofilm forming capabilities of CF *P. aeruginosa* isolates and pH of growth media. Graphs show the tendencies for a relationship for each isolate – some of them show negative relationship (CFPA5, CFPA7, CFPA11, CFPA17 and PAK) while the others exhibit positive correlation (CFPA1, CFPA4, CFPA12 and CFPA16).

➢ The correlation analysis also estimates the squared correlation coefficient (R^2) which is an indicator of the variance in biofilm forming capabilities that might be explained (at least, theoretically) by the pH variance. Despite their pilot nature the established tendencies warrant further investigations

Conclusions & Future Work

- Despite some clonal similarity, biofilm formation was variable and strain dependent but NaCl (0.5% w/v) and glucose (1% & 2% w/v) induce the process in some aerobically. Anaerobic conditions appear to reduce biofilm development.
- Some isolates became more adherent as pH decreased (e.g. CFPA11 in TSB + 1% glucose), while others favored neutral to slightly acidic or basic growth conditions.
- More investigations characterising a larger group of isolates may determine a true role of pH in influencing biofilm development of *P. aeruginosa* in the CF lung.

[1] Stepanovic *et al.*, J. Microbiol. Methods, 2000; 40:175-179