On Feasibility of Fluorescence-Based Bacteria Presence Quantification: *P.aeruginosa*

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Introduction

Objectives:

- Investigate the possibility of fluorescence-based quantification of bacterial presence for *P.aeruginosa*

Background:

- Wound healing normally occurs in the presence of bacteria, at levels ranging from contamination to colonization to infection.
- Existing clinical bacteria load assessment methods (biopsy or swabbing in combination with culture methods) are slow, labor- and time-consuming.
- Some clinically relevant bacteria produce fluorescent compounds
- Thus, fluorescence imaging/spectroscopy can be a modality for rapid assessment of bacteria load *in vivo.*
- *Pseudomonas aeruginosa* is a known pathogen implicated in numerous healthcare-associated infections and is known to express fluorescent metabolites during proliferation.

METHODS

PA01 was chosen as the representative strain for this study

A. Inoculum Parameters	
Test microorganism	*Pseudomonas aeruginosa (PA01)*
Growth media	3 gL^{-1} Tryptic Soy Broth (TSB)
Incubation period	16-20 hours (Overnight)
Incubation temperature	37 °C
Rinse solution	1x Phosphate Buffered Saline (PBS)
Centrifuge duration	4 min at 9000 x g
Expected inoculum load	10^5-10^7

B. Microbial Reference Quantification	
Quantification Method	Spot plate technique
Quantification period	Immediately after rinsing cells
Maximum dilution factor	10^7
Growth media	3 gL^{-1} Tryptic Soy Agar (TSA)
Incubation temperature	25 °C (Room Temperature)
Incubation period	7 days

Microbial Rapid Quantification

For the rapid quantification of PA01 within liquid media, optical density (OD) measurements at 600nm were calibrated to CFU counts obtained from microbial reference quantification

OD-correlated Fluorescence Spectroscopy

3mL macro cuvette with 400nm as the excitation wavelength and 420-520nm as emission range (LS50B Luminescence Spectrometer, Perkin-Elmer Ltd.)

$$fl = \int_{420}^{520} \Phi(\lambda) d\lambda$$

Pre-processing

Cuvettes with a sterile TSB solution demonstrate autofluorescence while excited at 400nm. To take into account substrate autofluorescence, we used a ratio

$$FL = \frac{fl - fl_s}{fl_s}$$

RESULTS

Calibration procedure shows the following dependence between bacteria concentration N (CFU/mL) and optical density OD (R^2=0.991):

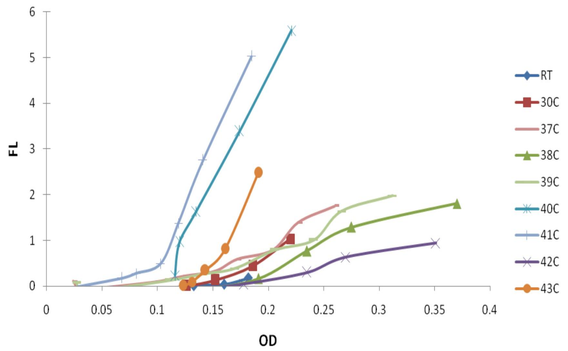
$$N = (5 * 10^9 * OD)^{0.97}$$

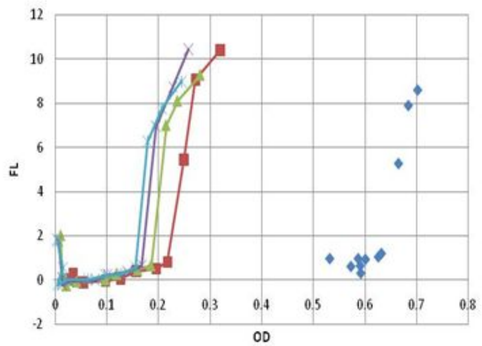
We have performed three types of experiments:

1. dependence of bacterial growth and fluorescence on temperature
2. dependence of bacterial growth and fluorescence on inoculum concentration
3. dependence of bacterial growth and fluorescence on initial nutrient concentration

1. Dependence on temperature:

The growth and fluorescence of *P.aeruginosa* were investigated at various temperatures: room temperature (RT), 30, 37, 38, 39, 40, 41, 42, and 43C. The initial nutrients concentration was 3 g/L.



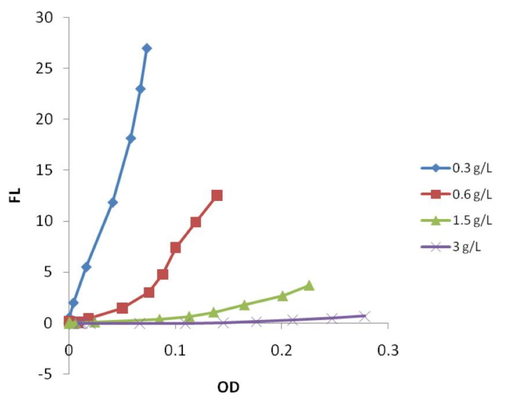


2. Dependence on Inoculum Concentration:

The growth and fluorescence of *P.aeruginosa* were investigated for various inoculum concentrations at 38C. Inoculum concentrations: original stock (blue rhombs ◊), 1/20 (red, squares □), 1/40 (green, triangles Δ), 1/60 (purple, cross x), 1/80 (blue, x). The initial nutrient's concentration was 3 g/L.

3. **Dependence on Initial Nutrients Concentration:**

The growth and fluorescence of *P.aeruginosa* were investigated for various initial nutrients' concentrations: 0.3, 0.6, 1.5, and 3 g/L. Temperature was held at 38C.



DISCUSSION

- We have performed simultaneous measurements of optical density (OD600) and fluorescence of *P.aeruginosa* (PA01) in media, which allow direct correlation between fluorescence and bacterial concentration.
- Pyoverdine production is affected by numerous factors, including ambient temperature, inoculum concentration, and initial nutrients concentration
- In nutrient-rich media (1.5 and 3.0 g/L) bacteria demonstrate delayed production of pyoverdine
- In nutrient-poor media (0.3 and 0.6 g/L) bacteria start producing pyoverdine almost immediately
- Other caveats:
  - Pyoverdine fluorescence is affected by two additional factors [1]: a) iron bound to pyoverdine quenches fluorescence, b) pyoverdine production is affected by iron availability. Thus, *P.aerugenosa* fluorescence can be diminished near blood vessels.
  - *P. aeruginosa* isolated from acute infections differs substantially in phenotype from those isolated from chronic infections [2]
  - *P.aeruginosa* can sequester the iron in ways other than pyoverdine production [3]
  - Fluorescence in the tissue can differ significantly from experiments in the media. It was estimated [4] that for pyoverdine fluorescence the correction factor can be in the range of 2-2.25
- In future work, we plan to investigate feasibility of bacterial load quantification for another model: porphyrins production by *S.aureus*.

REFERENCES

1. Meyer JM, Abdallah MA (1978) The Fluorescent Pigment of Pseudomonas fluorescens: Biosynthesis, Purification and Physicochemical Properties. J Gen Microbiol 107:319–328
2. Smith EE, Buckley DG, Wu Z et al. (2006) Genetic adaptation by Pseudomonas aeruginosa to the airways of cystic fibrosis patients. P Natl Acad Sci USA 103: 8487–8492
3. Cornelis P., and Matthijs S. (2002) Diversity of siderophore-mediated iron uptake systems in fluorescent pseudomonads: not only pyoverdines. Environ. Microbiol. 4, 787–7898
4. Saiko G, Douplik A, Extraction of Intrinsic Fluorescence in Fluorescence Imaging of Turbid Tissues, in Proc. BioImaging 2020, Valletta, 2020

CONCLUSIONS

- *P.aeruginosa* is a versatile and opportunistic microorganism. It remains metabolically active even at 43C.
- Pyoverdine production is affected by numerous factors, including ambient temperature, inoculum concentration, and nutrients availability.
- Feasibility of *in vivo P.aeruginosa* load quantification seems problematic at this point. Further experiments are required.

CONTACT INFORMATION

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