P3.37 - FAST AND SENSITIVE DETECTION OF *PSEUDOMONAS AERUGINOSA* IN CLINICAL SETTINGS USING ENGINEERED REPORTER PHAGES

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ABSTRACT

P. aeruginosa is a bacterial pathogen responsible for a wide range of infections. As a result, the World Health Organization identified it as one of the top priority pathogens that urgently calls for the development of novel treatments. Bacteriophages have emerged as a promising therapeutic approach and their properties can be enhanced by phage-engineering. This opens an extensive variety of possibilities, allowing to assemble chimeric phages with new functions. Considering the slow turnover of conventional diagnostic methods and the problems associated with the molecular and immunogenic methods, this study aimed at assembling a bioluminescence-based reporter phage for the fast and sensitive detection of *P. aeruginosa* in clinical care.

Using the yeast-based phage engineering platform, the phage vB_PaeP_PE3 was genetically modified by removing genes with unknown function (*g*1-*g*12) and then used as a scaffold for the insertion of the NanoLuc® luciferase gene that was swapped with gene *g*53. The assembled reporter phage (vB_PaeP_PE3Δgp1-*gp*12,*gp*53:NLuc) was then used for sensitivity and specificity assays. The detection limit was evaluated through the infection of serial dilutions of *P. aeruginosa* suspensions with the reporter phage, and subsequent quantification of luminescence.

Our data showed that the assembled reporter phage was capable of reliably detect 500 CFU/mL within 7h or an average 1 CFU/mL after 24h, and no false positives were observed. Similar results were also obtained when the reporter phage was tested in blood, being capable of detecting an average of 8 CFU/mL within 24 hours.

Overall, compared to culture-dependent methods, the NanoLuc-based reporter phage allows a fast and sensitive detection of *P. aeruginosa* cells using a simple protocol. Therefore, this phage-based detection system is a promising alternative to the common methods for the accurate detection of *P. aeruginosa* in clinical settings.

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