VERSATILE ENGINEERING OF LYSINS: ONE DROP TO KILL

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ABSTRACT

The rapid emergence and spread of antimicrobial resistance has evolved to a major health care threat, which is further exacerbated by the diminished antibiotic development pipeline [1]. Lysins are a novel class of enzyme-based antibiotics. A portfolio review in 2016 ranked lysins, based on their clinical impact and technical feasibility, as the alternative class of antibiotics with the greatest potential [2]. Moreover, several lysins are currently under evaluation in clinical trials [3]. Here, we here report on the development of a novel microdroplet-based screening platform that can be used to identify customized lysins for each multi-drug resistant bacterium.

KEYWORDS: Ultra-high-throughput droplet microfluidics, Multi-drug resistant bacteria, DNA shuffling, Lysin engineering, Miniaturized lysis assay

INTRODUCTION

A unique feature of lysins is their modular structure, providing opportunities to customize lysins towards every patient's bacterial isolate. However, the current progress is empirical and hampered by tedious cloning procedures. To address this technical barrier, we developed a novel DNA shuffling method, coined VersaTile, enabling the generation of up to millions of variants in a single reaction. Using an in-house microtiter-based screening method, we established a hit-to-lead development process for engineered lysins, similar to the development of pharmaceutical drugs (manuscript in preparation). Still, this workflow only allowed the screening of than 0.01% of the total possible variants, clearly indicating the need for a more high-throughput screening method [4].

RESULTS AND DISCUSSION

We here combine VersaTile with a custom developed ultra-high-throughput microfluidics-based screening workflow (Figure 1), facilitating the screening of the entire protein landscape. This platform consists of three main modules: DNA amplification, lysin expression and selection of variants with antibacterial activity. The different modules of the platform are fabricated using standard PDMS soft lithography techniques.

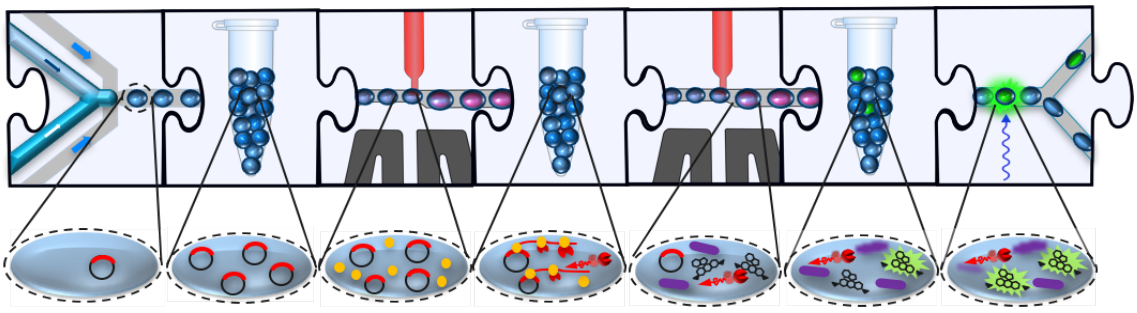

Figure 1: Schematic workflow of the high-throughput, droplet-based microfluidic platform for screening engineered lysin variants. The microfluidic unit operations are represented on top, and the effect of the operation on the content of individual droplets is shown below. At first, each engineered lysin (DNA) is separately encapsulated in a microfluidic droplet, where it is amplified. Pico-injecting an expression mixture in the droplets, ensures that each variant is produced. The antibacterial activity of each variant is assessed by adding bacteria, and lysis of the bacteria will result in a fluorescent signal. Finally, this fluorescent signal is used to sort out the active variant of which the specific structure is determined by sequencing in order to derive structure-activity-relationships that form the basis for the next engineering round.

In the first module, a DNA library of engineered lysins is encapsulated inside 15 pL droplets according to Poisson statistics (Figure 2A), and each variant is amplified by rolling circle amplification (RCA) (Figure 2B). In the second module, the expression of the engineered lysins is initiated by pico-injecting an *in vitro* transcription and translation mixture in each droplet. In a third module, bacteria are injected into the droplets to assess the antibacterial activity of the expressed lysins. Cell lysis activates fluorescence, correlating efficient lysis to a readable fluorescence signal. The latter is used to sort out the fluorescent droplets out of the pool of negative variants.

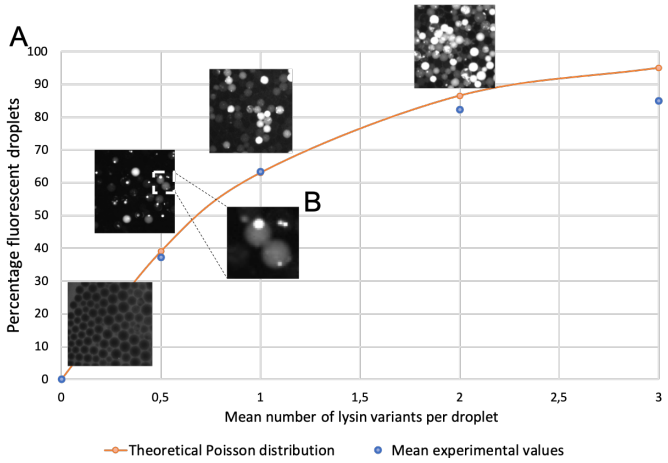

Figure 2: Comparison of theoretical Poisson distribution to our encapsulation of a DNA library of engineered lysins. (A) The encapsulation of our engineered lysins (blue dots) follows the predict Poisson distribution (orange curve). (B) After amplification of the engineered lysin, a clear DNA blob is seen. The DNA was stained using Sybr Green.

Via sequencing we then determine the building blocks of the engineered lysin. With this information we derive the structure-activity-relationships (SARs), forming the basis for the next engineering round.

CONCLUSION

In summary, we have demonstrated a microfluidic approach to derive SARs from active engineered lysins. We are now further characterizing these new engineered lysins regarding their antibacterial spectrum, stability, working mechanism, etc. The combination of VersaTile and the microfluidics-based platform represents a key breakthrough for high-throughput lysin engineering and selection, and will facilitate the development of new designer lysins.

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