A novel thermostable YtnP lactonase inhibits biofilm formation and induces decomposition of preformed *Pseudomonas aeruginosa* biofilms

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INTRODUCTION: Biofilm-associated infections are the main cause of biomaterial implant failure today. The increasing prevalence of antibiotic-resistant pathogens often results in the only solution of implant movement, with serious consequences for patients. Recently, various antimicrobial agents have been recognized as a promising strategy to prevent biofilm formation on implant surfaces [1]. Quorum sensing (QS) plays a central role in the control of bacterial virulence and biofilm formation. The use of quorum quenching (QQ) enzymes to target QS is therefore a promising innovative approach for the development of enzyme-based antivirulence therapeutics, which represent a potential solution to combat infections caused by multidrug-resistant pathogens. This study aimed to characterize the novel YtnP lactonase from the clinical isolate *Stenotrophomonas maltophilia* 6960 and to investigate its potential to combat the virulence of multidrug-resistant (MDR) *Pseudomonas aeruginosa* MMA83.

EXPERIMENTAL: The biochemical analysis (pH and thermostability) of the purified recombinant YtnP lactonase (final concentration 50 µg/ml) was conducted with well-diffusion assay using *Chromobacterium subtsugae* CV026 as a biosensor strain with 10 µM *N*-octanoyl-L-Homoserine lactone (C8-HSL quorum sensing signaling molecule) as a substrate [2]. The pH stability of recombinant YtnP lactonase was analyzed in a pH range of 4 to 9 (pH interval of 0.5) using appropriate buffers. Recombinant YtnP lactonase was preincubated for 1h at temperatures ranging from 30 to 100 °C with an interval of 10 °C. Multiple alignments of amino acid sequences of YtnP lactonase with other close clustered functionally characterized lactonases were performed using ClustalW software (https://www.ebi.ac.uk/Tools/msa/clustalo/) and Espript 30 (http://espript.ibcp.fr). The effect of recombinant YtnP lactonase on *P. aeruginosa* MMA83 biofilm formation and decomposition was monitored using fluorescence microscopy [2].

RESULTS AND DISCUSSION: The recombinant YtnP lactonase retained its almost complete activity after exposure to temperatures ranging from 30 to 100 °C. The thermostability of YtnP lactonase can be explained by the absence of the N-terminal 63 amino acids found in both YtnP and thermostable lactonase from *Bacillus licheniformis*, and by the possible role of the N-terminal segment in disrupting the spherical organization of the proteins, possibly affecting their thermostability. YtnP lactonase exhibits broad pH stability, with optimal enzyme activity observed between pH 6 and 8 at 30°C, peaking at pH 7. In addition, significant enzyme activity was also maintained at lower pH values. Fluorescence microscopy shows the strong effect of YtnP lactonase in preventing the formation of *P. aeruginosa* MMA83 biofilms and initiating the decomposition of preformed biofilms [2].

CONCLUSIONS: Overall, advantageous biochemical properties, such as high temperature and pH stability makes YtnP lactonase a potential anti-biofilm agent that could be used for the coating of medical implants and for the design of innovative therapeutics to combat bacterial infections caused by MDR *P. aeruginosa***.**

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