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TOPIC(s) : Enzyme discovery and engineering

Engineering enzymes to enhance lactonase activity towards bacterial quorum sensing control

AUTHORS

Raphael BILLOT / GENE&GREENTK, 19-21 BOULEVARD JEAN MOULIN, MARSEILLE
Corresponding author : David DAUDE / david.daude@gene-greentk.com

PURPOSE OF THE ABSTRACT

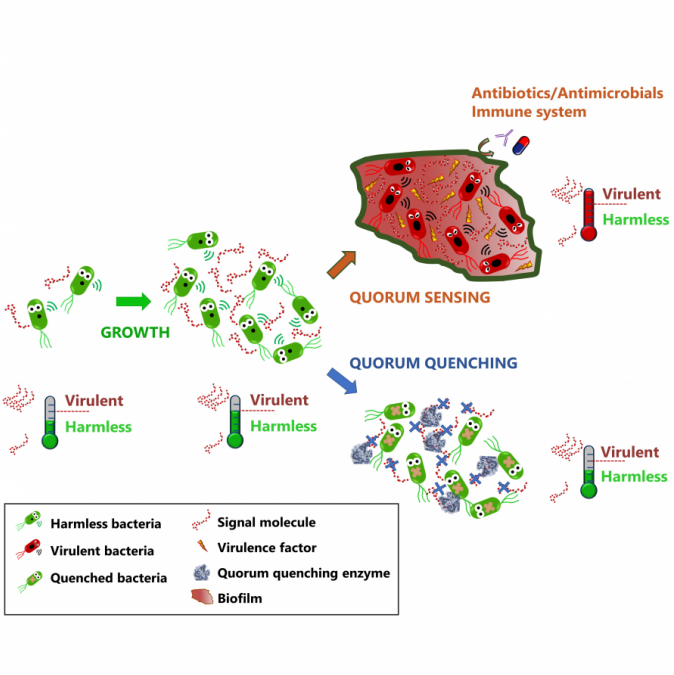
Many bacteria use quorum sensing (QS), a bacterial communication system based on the diffusion and perception of small signaling molecules, to synchronize their behavior in a cell-density dependent manner. This mechanism enables bacteria to adapt their behavior according to their population size and regulate the expression of genes involved in virulence, antimicrobial resistance and biofilm formation. Methods have emerged to inhibit bacterial communication and counteract its noxious traits. Chemical inhibitors, sequestering antibodies and degrading enzymes have been developed and proved efficient to decrease bacterial virulence. Among these, enzymes from various origins having lactonase or acylase activities were particularly studied for their ability to degrade acyl-homoserine lactones (AHL) involved in the communication of numerous Gram-negative bacteria.

Our work focuses on the extremophilic lactonase SsoPox able to degrade a wide panel of AHL. This enzyme has a natural preference for the hydrolysis of AHL with long acyl chains (>C8) and a relatively low activity for short-chain AHL while these latter are widely involved in the QS of numerous pathogens. Enzyme engineering was applied to increase the performance of SsoPox towards various types of AHL. Structural analysis led to the identification of a mobile loop surrounding the active site that highly impacts the lactonase activity spectrum. Combining alanine scanning, saturation mutagenesis and semi-rational approaches, variant libraries were generated and improved variants with up to 297-fold activity increase towards short chain AHL were isolated. Tridimensional structures of these variants revealed rearrangement of the active site cavity leading to the reorientation of lactone acyl chain in favor of small AHL recognition.

The potential of the best identified variant to disrupt short-AHL based QS was then studied in vitro towards the bacterium Serratia sp. 39006, which mainly uses C4 AHL. A multi-faceted approach combining phenotypic, proteomic and metabolomic analyses allowed to demonstrate the ability of the enzyme to drastically alter strain behavior by disrupting various phenotypes including biofilm formation, production of antibiotics, mobility and floatability.

Development of these more effective QS disrupting enzymes will pave the way for developing concrete biotechnological applications to tackle the virulence of bacterial pathogens affecting animal nutrition, agriculture and human health.

FIGURES



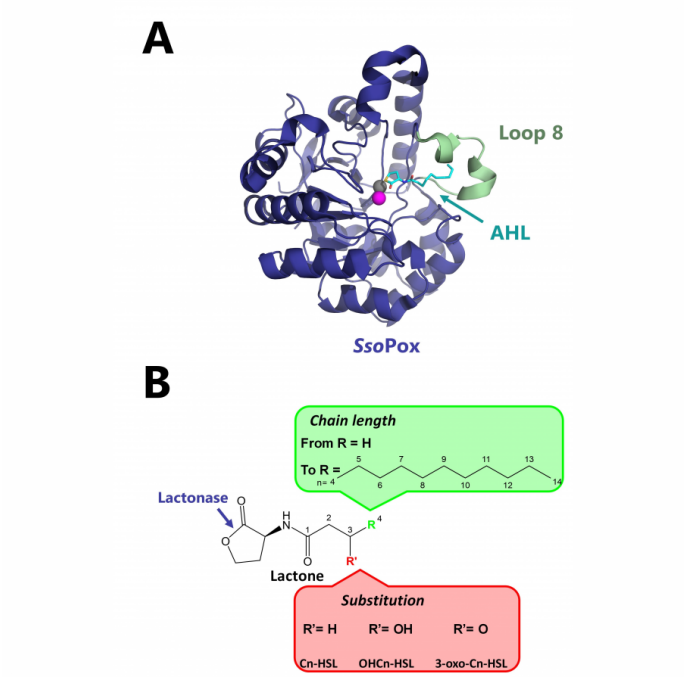


FIGURE 1

Disrupting bacterial quorum sensing (QS) with enzymes: a promising approach against pathogens. Bacteria secrete diffusible molecules during growth. When the concentration reaches a threshold, bacteria synchronize the expression of genes involved in virulence and biofilm. Enzymes able to degrade signal molecules disrupt QS and keep bacteria harmless

FIGURE 2

The SsoPox enzyme and its various AHL substrates. (A) Tridimensional structure of SsoPox (dark blue), in complex with an AHL (cyan). The mobile loop (loop 8) is highlighted in green. (B) General structure of QS AHL (HSL, oxo-HSL and OH-HSL). Lactonases degrade AHL by opening lactone rings.

KEYWORDS

quorum sensing | quorum quenching | lactonase | enzyme engineering

BIBLIOGRAPHY