Fast, Continuous, and High-Throughput (Bio)Chemical Activity Assay for N-Acyl-l-Homoserine Lactone Quorum-Quenching Enzymes.

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Abstract

Quorum sensing, the bacterial cell-cell communication by small molecules, controls important processes such as infection and biofilm formation. Therefore, it is a promising target with several therapeutic and technical applications besides its significant ecological relevance. Enzymes inactivating N-acyl-l-homoserine lactones, the most common class of communication molecules among Gram-negative proteobacteria, mainly belong to the groups of quorum-quenching lactonases or quorum-quenching acylases. However, identification, characterization, and optimization of these valuable biocatalysts are based on a very limited number of fundamentally different methods with their respective strengths and weaknesses. Here, a (bio)chemical activity assay is described, which perfectly complements the other methods in this field. It enables continuous and high-throughput activity measurements of purified and unpurified quorum-quenching enzymes within several minutes. For this, the reaction products released by quorum-quenching lactonases and quorum-quenching acylases are converted either by a secondary

enzyme or by autohydrolysis to l-homoserine. In turn, l-homoserine is detected by the previously described calcein assay, which is sensitive to α-amino acids with free N and C termini. Besides its establishment, the method was applied to the characterization of three previously undescribed quorum-quenching lactonases and variants thereof and to the identification of quorum-quenching acylase-expressing Escherichia coli clones in an artificial library. Furthermore, this study indicates that porcine aminoacylase 1 is not active toward N-acyl-l-homoserine lactones as published previously but instead converts the autohydrolysis product N-acyl-l-homoserine.

Leibniz-HKI-Autor*innen

Daniel Last

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