TITLE: CONSTRUCTION OF A MODULAR DEVICE FOR AUTONOMOUS CONTROL OF GENE EXPRESSION IN INDUSTRIAL BACTERIAL STRAINS

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ABSTRACT:

Current methods available for induction of gene expression in genetically engineered bacteria require addition of inducing compounds to the culture medium (IPTG, xylose and arabinose), which is undesirable for industrial strains due to additional costs to the production process. As an alternative, we constructed a regulatory network that leads the producer to self-monitor and autonomously induce protein synthesis at desired cell density. The autoinduction device for gene expression was developed and optimized for *Bacillus subtilis* based on the *lux* quorum-sensing system of *Vibrio fischeri*. Device optimization began with separating the induction (*luxR* and *luxI*) and the response (promoter and target gene) modules in the genome. Decoupling allows separate optimization of promoters, and gene of interest to be positioned at any locus in the genome regardless of the location of the autoinduction module. Therefore, the *lux* promoter (P*lux*) located between the *luxR* and *luxI* genes, was duplicated. Ten variations of P*lux* were constructed and tested. Optimized P*lux* generated a range of promoter strengths, and the best version increased the level of gene expression 117 times compared to the wild type. Moreover, the best device configuration showed a 51-fold activation of gene expression. Autonomous induction of gene expression is triggered during the early log phase of growth, and the maximal expression rate is reached during transition from the late log to the stationary phase. Exchanging the *luxABCDE* operon for *gfp* as reporter did not affect gene expression pattern under control of the autoinduction device. Neither using different *B. subtilis* strains does. Finally, none of the device configurations affected the culture growth rate or the final cell density. We have built a stable, highly modular, and decoupled device for autoinduction of gene expression, which do not cause metabolic burden to the cell.

Keywords: Synthetic biology, quorum-sensing, autoinduction, gene expression, *Bacillus subtilis*

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