THE UNIVERSITY OF HONG KONG

HKU iGEM 2012

Pseudokill

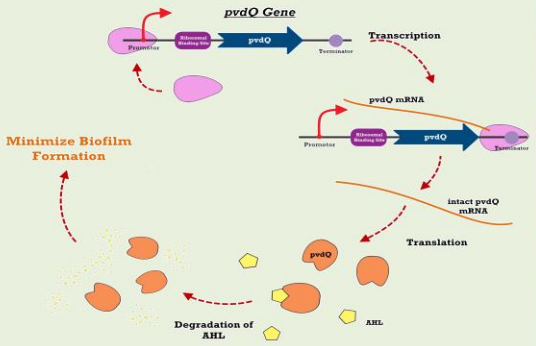
Quorum Sensing Disruption through pvdQ Production

Abstract

Planktonic, motile bacterial cells are unable to survive when subject to a strong immune response or antibiotic selection. It is advantageous, however, for bacteria to irreversibly attach to a particular surface and communicate through acyl-homoserine lactone (AHL) signaling, forming highly persistent biofilm structures. The metamorphosis of planktonic bacteria into a biofilm phenotype allows certain genes to be expressed. This is responsible for bacterial pathogenicity as biofilm-forming bacteria can withstand antibiotic concentrations that would otherwise be fatal.

HKU's iGEM team aims to develop a simple and effective method of inhibiting biofilm formation. We engineered Escherichia coli to harbor an AHL-degrading genetic system, which involves the expression of the pvdQ enzyme. PvdQ is a quorum-quenching acylase that functions to degrade long-chain AHL molecules), e.g. 3-oxo-C12-HSL, that bacteria like Pseudomonas aeruginosa utilize for biofilm formation. If pvdQ is successfully produced, it can become a potential strategy to disrupt quorum sensing and a medical treatment for chronic infections that result.

We were able to successfully amplify the functional pvdQ gene from P.aeruginosa genomic DNA, and express it in the pET21a commercial expression vector. Then, from the colorimetric AHL assay that measures the concentration of ester bonds in the intact AHL molecule, we were able to conclude that pvdQ displays acylase activity towards C-12 AHL molecules.



AIM

Once biofilms form on a surface and produce extracellular materials to defend themselves, it is very difficult for one's body or external drugs to eradicate them. Recognizing this problem, HKU's iGEM team aims to tackle the very mechanism of quorum sensing that is critical to biofilm formation. Taking this approach, we can reduce the problem of biofilm formation at its roots.

Project Flow

pvdQ gene was amplified from the genome of *Pseudomonas aeruginosa*.
HisTag sequence was added to the reverse primer containing the standard suffix; while the forward primer consisted of the standard prefix.
Final product is pvdQ gene with a HisTag sequence attached to the C-terminal of the β chain.
[The HisTag sequence is necessary for confirming pvdQ protein expression and subsequent HisTag-specific purification of the protein].

↓

Overlap extension site-directed mutagenesis was carried out to yield a pvdQ gene with a silent mutation at the illegal PstI site at 1,491bp. *[This does not change the function of the pvdQ protein, as the sequence of amino acids remains the same.]*

The PstI Site, CTGCAG, encodes the amino acids leucine and glutamine respectively. Thus, a point mutagenesis at the Leucine codon, CTG > CTC, would alter the PstI sequence recognized by the restriction enzyme. Similarly, the Glutamine codon can be altered, CAG -> CAA.

↓

Two oligonucleotide primers, complementary to one another and containing the deletion site, were used in addition to the standard prefix and suffix primers.
First step: Requires amplification using the standard prefix and one of the oligonucleotide primers for one reaction, and the standard suffix with the other oligonucleotide primer for a second reaction.
Second step: All four primers were added to the reaction mixture to synthesize a full-length double stranded product.
Last step: Uses only the prefix and suffix to amplify the desired gene with the mutation.

↓

The pvdQ gene was inserted into an expression vector, pET21a, and be transformed into the BL21 expression host.
Under the control of T7 promoter, pvdQ gene is transcribed by T7 polymerase upon IPTG induction, subsequent translation in the host cell produces the **functional pvdQ enzyme.**

↓

SDS gel electrophoresis and Western blot using an anti-HisTag primary antibody was used to detect the presence of the expressed pvdQ protein.
The enzymatic activity of pvdQ towards C12-HSL was examined by a colorimetric method using an AHL assay.

Result

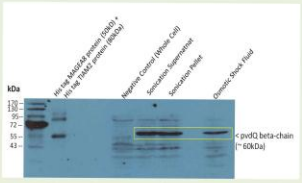


Figure 1: Developed X-ray Film of Western Blot Transfer to Evaluate pvdQ Expression
The pvdQ protein is subject to posttranslational processing resulting in its autocatalytic cleavage into an alpha subunit (18kDa) and a beta subunit (60kDa). As the alpha subunit does not contain the HisTag, its expression is not visible through this method. The positive controls are two HisTag standard proteins of 50 and 80 kDa. The negative control is the IPTG induced BL21 without the plasmid (the whole cell bacterium). As the 60kDa band is absent from the negative control lane but present in the sonication supernatant and pellet, the pvdQ protein was successfully expressed in the commercial vector and exits in both a soluble and insoluble state. Moreover, the osmotic shock fraction that is the periplasmic portion of the cell also exhibits a 60kDa band. This confirms that the signal peptide on pvdQ functions to translocate it to the periplasm of the host cell as well.

In each of the graphs, PBS is used as a negative control to monitor the natural degradation of AHL in the absence of an enzyme. Boiling of each of the respective samples denatures the proteins and decreases their activity as an AHL acylase. Therefore, compared to the unboiled, intact samples, more AHL remained after 4 hours of incubation in the boiled samples for each graph.

A greater degree of AHL degradation was observed for the sonication supernatant than the pellet. This might imply that most of the pvdQ enzyme is present in the soluble form or the activity of pvdQ in the supernatant is greater.
While the greatest amount of AHL degradation is observed for the whole cell, this does not correlate to pvdQ enzyme activity or its amount as the enzyme is not directly in contact with AHL (like the supernatant and pellet fractions) because of the intact cell membrane.

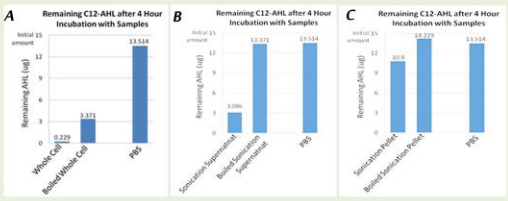


Figure 2: *A. Remaining AHL after 4 Hours of Incubation for Whole Cell and Boiled Whole Cell BL21 Samples with the Expression Vector. B. Remaining AHL after 4 hours of Incubation for Sonication Supernatant and the Boiled Sonication Supernatant BL21 Samples with the Expression Vector. C. Remaining AHL after 4 hours of Incubation for Sonication Pellet and the Boiled Sonication Pellet BL21 Samples with the Expression Vector*

Future Directions

1. Characterize the BBa_K1588001 biobrick by inducing the expression of pvdQ. Then, compare the efficiency of pvdQ expression by measuring its potential to degrade C-12 AHL.
2. Construct a biobrick with a strong constitutive promoter, BBa_J23119, eliciting transcription of the pvdQ gene. This promoter is a strong promoter and its expression is independent of environmental and developmental (endogenous) factors. Thereby, it will ensure a ubiquitous, baseline expression of pvdQ that will be dependent on the cell density of E.coli. Characterize this biobrick by checking for the expression of pvdQ and its ability to degrade C-12 AHL molecules.
3. Compare the potential of AHL degradation between the BBa_K1588001 biobrick and the potential constitutive promoter – pvdQ one.
4. Construction of a system in which pvdQ expression dependent on another factor of cell density but independent of AHL. If pvdQ is dependent on AHL, then when AHL levels decreases, the pvdQ expression level will decrease accordingly. Such a negative feedback system leads to the re-accumulation of AHL, re-establishing biofilm formation through quorum sensing.
5. A signal peptide that directs pvdQ from the periplasmic space into the external environment should be added at the 5'-end of the pvdQ gene sequence. This will enhance the enzyme's efficacy, as its secretion will permit direct interaction with and degradation of the C-12 AHL molecules.
6. The synthetic E.coli, used to produce pvdQ, can be immobilized into small protein-delivery bores. These structures can be stimulated to efficiently release pvdQ at the desired location, mimicking conventional drug-delivery systems. Encapsulation using 2% alignate is most desirable because no internal enzyme is able to digest this polymer and the resulting bead maintains a constant shape and boundary.

Article Publishing

To establish a stronger presence in HKU, we published an article in HKU's Undergraduate Magazine, which was freely distributed to all HKU undergraduates. The article introduces the current workings and future implications of synthetic biology, as well as the HKU iGEM Team and the competition.

Street Drama

A brand new approach-Street Drama is used thid year, to get rid of the routine and tedious works and go into the crowds!
Through our casts, 'Synthia' the bacterium, 'Dr. Venter' the scientist, the Moralist, the laboratory helper, and their interaction with common public, the definition and purposes of synthetic biology were accurately illustrated. Also, as the Moralist and Dr. Venter sparked a heated debate, our audience acquired a glimpse into the dilemma that technology and ethics are facing today.

Video Broadcasting

As animations and videos are always attractive and impressive to people, we include our video covering Synthetic Biology and the iGEM competition into the University's daily broadcasting schedule. The video clip includes simple illustration of biobrick production and the application of synthetic biology. Audiences of differing fields of study, regardless of their background in Synthetic Biology, could understand the concept after viewing TVs around the campus. We hope this would also bring forth some capable undergraduates for next year's recruitment of HKU's iGEM team.

Conclusion

1. Successful cloning of pvdQ gene from the Pseudomonas aeruginosa genome.
2. Successful silent site-directed mutation of the PstI site within the pvdQ gene.
3. From the AHL assay, it can be concluded that expression of a functional pvdQ protein displays acylase activity that degrades C-12 AHL.
4. Biobricks Submitted:
->BBa_K855000: PvdQ containing the Illegal PstI site and HisTag sequence at the C-terminal. Ligated to the double terminator, BBa_B0015.
->BBa_K855005: Leucine amino acid of the PstI site is mutated in pvdQ of part BBa_K855000.
->BBa_K855006: Glycine amino acid of the PstI site is mutated in pvdQ of part BBa_K855000.
->BBa_K855008: AHL inducible production of pvdQ with the Leucine codon of the PstI site mutated. Consists part of BBa_K137076.
->BBa_K855009: AHL inducible production of pvdQ with the Glycine codon of the PstI site mutated. Consists part of BBa_K137076.
->Bba_K855002: B0015-R0040-B0032 Composite Part

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Faculty of Engineering,
The University of Hong Kong