Analyzing DNA Sequences for Quorum Sensing Inhibition

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INTRODUCTION

The problem? Biofilms.

Biofilms are complex, interdependent communities of surface-associated bacteria. The microorganisms are enclosed in a matrix that can occur on any surface, in particular aquatic and industrial water systems. Because they often form sessile communities on medical devices and food preparation settings, millions of people in the developed world are affected by diseases such as cystic fibrosis and pneumonia, with some experiencing death as a consequence.

Possible solutions? Inhibit communication.

For the safety of both plants and animals, it is vital to be able to eliminate biofilms. However, the polymers in the matrix of a biofilm retard diffusion of antibiotics, giving them an increased tolerance to immunological defenses, stress, and biocides. There is also a greater frequency for individual bacteria in biofilms to develop antibiotic resistance due to a higher probability of horizontal gene transfer among bacteria in close proximity. A biofilms' resistance to antibacterial agents warrants the need to either:

1. Prevent initial growth. 2. Sterilize the colony. **3. Inhibit communication.**

How? Analyze bacterial sequences for LuxR protein.

Quorum sensing is the mechanism by which bacteria "communicate." The seaweed *Delisea pulchra* has stayed slime-free in bacteria-infested waters by naturally preventing quorum sensing through the use of furanones, compounds structured similarly to an AHL signaling molecule in Gram negative bacteria. In high enough concentrations, furanones displace AHL from binding to its quorum-sensing signal receptor. In this study, we seek to analyze bacteria with the AHL signaling molecule and LuxR protein to accurately produce alternate furanone variations, and ultimately prevent bacterial communication.

METHODS

1.Find the LuxR protein sequence, which produces the AHL signaling molecule, through Uniprot, a comprehensive and freely accessible database of protein sequence and functional information.

```
          10         20         30         40         50
MKNINADDTY RIINKIKACR SNNDINQCLS DMTKMVHCEY YLLAIIYPHS
          60         70         80         90        100
MVKSDISILD NYPKKWRQYY DDANLIKYDP IVDYSNSNHS PINWNIFENN
         110        120        130        140        150
AVNKKSPNVI KEAKTSGLIT GFSFPIHTAN NGFGMLSFAH SEKDNYIDSL
         160        170        180        190        200
FLHACMNIPL IVPSLVDNYR KINIANNKSN NDLTKREKEC LAWACEGKSS
         210        220        230        240        250
WDISKILGCS ERTVTFHLTN AQMKLNTTNR CQSISKAILT GAIDCPYFKN
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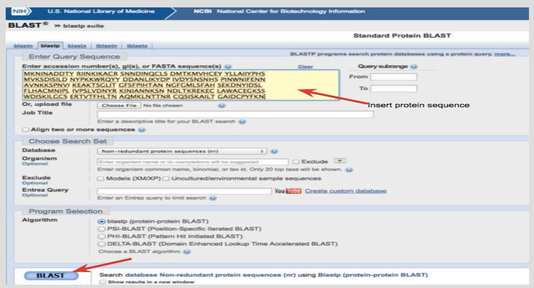
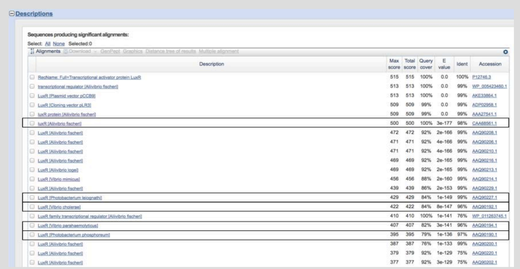
2. Utilize Stanford Protein BLAST on the National Center for Biotechnology Information website to find species of bacteria with the LuxR protein sequence.



3. Choose five bacteria with the LuxR sequence and an Ident of over 95% sequence similarity. In this case, the bacteria: 1. Aliivibrio fischeri, 2. Photobacterium leiognathi, 3. Vibrio cholerae, 4. Vibrio parahaemolyticus, 5. Photobacterium phosphoreum. Perform the tests indicated in "Results section."



RESULTS

1. Amino Acid Sequencing

Analyze the amino acid sequence differences to see how to precisely plan a variant furanone.

```
Photobacterium_phosphoreum      MGMKDINADDTYRIINKIKACRSNNDINQCLSDMTKMVHCEYYL-LAIMY
Photobacterium_leiognathi       MGMKDINADDTYRIINKIKACRSNNDINQCLSDMTKMVHCEYYL-LAIIY
Vibrio_cholerae                 MGMKDINADDTYRIINKIKACRNNNDINQCLSDMTKMVHCEYYL-LAIIY
Vibrio_parahaemolyticus         MGMKDINADDTYRIINKIKRCRSNKDINQCLSDMTKMVHCEYYL-LAIIY
Aliivibrio_fischeri             --MKNINADDTYRIINKIKACR-AYDINQCLSDMTKMVHCEYYLTLAIIY
                                  **:************ **   ****************** ***:*

Photobacterium_phosphoreum      PHSMVKSDISILDNYPKKWRQYYDDANLIKYDPIVDYSNSNHSPINWNIF
Photobacterium_leiognathi       PHSMVKSDISILDNYPKKWRQYYDDANLIKYDPIVDYSNSNHSPINWNIF
Vibrio_cholerae                 PHSMVKSDISILDNSPKKWREYYDDANLIKYDPIVDYSNSNHSPINWNIF
Vibrio_parahaemolyticus         PHCMVKSDISIVDNYPKKWRQYYDDANLIKYDPIVDYSNSNHSPINWNIF
Aliivibrio_fischeri             PHSMVKSDISILDNYPKKWRQYYDDANLIKYDPIVDYSNSNHSPINWNIF
                                **.********:** *****:****************************

Photobacterium_phosphoreum      ENNAVNKKSPNVIKEAKTSGLITGFSFPIHTANNGFGMLSLAHSEKDNYI
Photobacterium_leiognathi       ENNAVNKKSPNVIKEAKTSGLITGFSFPIHTANNGFGMLSFAHSEKDNYI
Vibrio_cholerae                 ENNAVNKKSPNVIKEAKTSGLITGFSFPIHTANNGFGMLSFAHSEKDNYI
Vibrio_parahaemolyticus         ENNAVNKKSPNVIKEAKSSGLITGFSFPIHTANNGFGMLSFAHSEKDNYI
Aliivibrio_fischeri             ENNAVNKKSPNVIKEAKTSGLITGFSFPIHTANNGFGMLSFAHSEKDNYI
                                ***************:********************:*********

Photobacterium_phosphoreum      DSLFLHACMNIPLIVPSLVDNYRKINIANNKSNYDLXXREKECLAWACEG
Photobacterium_leiognathi       DSLFLHACMNIPLIVPSLVDNYXKINIANNKSNNDLTKREKECLAWACEG
Vibrio_cholerae                 DSLFLHACMNIPLIVPSLVDNYRKINIANNKSNTDLTKREKECLAWPCEG
Vibrio_parahaemolyticus         DSLFLQACMNIPLIVPSLVDNYRKINIANNKSNXDLTKREKECLAWACEG
Aliivibrio_fischeri             DSLFLHACMNIPLIVPSLVDNYRKINIANNKSNNDLTKREKECLAWACEG
                                *****:*************** ********** **  ********.***
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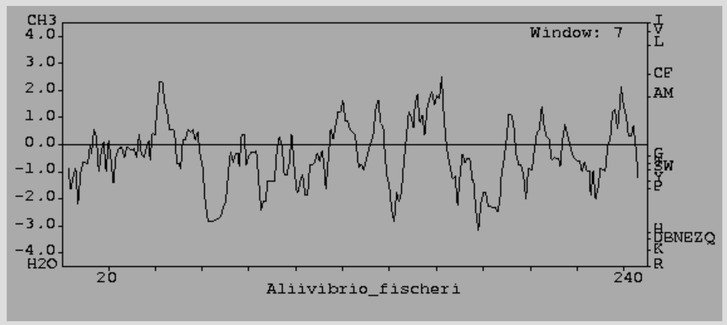
Amino acid sequences

Glycine – G	**Cysteine – C**	**Leucine – L**	**Methionine – M**
Threonine – T	**Valine – V**	**Phenylalanine – F**	**Tyrosine – Y**
Histidine – H	**Lysine – K**	**Arginine – R**	**Glutamine – Q**
Alanine – A	**Proline – P**	**Aspartic Acid – D**	**Asparagine – N**
Serine – S	**Tryptophan – W**	**Glutamic Acid – E**	**Isoleucine – I**

2. Hydrophobicity

The following graph indicates the hydrophobicity of Aliivibrio fischeri, with the degree of hydrophobicity shown above the line and hydrophilicity below the line.



3. Protein Folding Structure

Analysis of the alpha-helices, beta-strands, coils, and beta-turns for Allibrio fischeri. By applying this to all five bacteria, we may understand the way each sequence is folded to more precisely target each bacteria.

H - Alpha-helices
E - Beta-Strands
C - Coil
T - Beta-Turns

```
          10        20        30        40        50        60
....:....x....:....x....:....x....:....x....:....x....:....x
MKNINADDTYRIINKIKACRAYDINQCLSDMTKMVHCEYYLTLAIIYPHSMVKSDISILD     Aliivibrio_fischeri
CCCCCCCCEECCEHH CHHHHHHHHCCCCHHECCEEEEEEHEECEEEEECCCCCCCCCC     BPS
CCCH-HCCCCEEEEHEHHHHHH-ECCHH CHHHHEEHHEEEEEHEEECCCEEHEEEECCC     D_R
CCCCCCCCHHHHHHHHCCHHCCCCCCCCHHHHHHHHHEEEEEEECCCCCCCCEEEECCC     DSC
CCCCCCCCHHHHHHHHCCCEECCCCCEECCEEEEEECEEEEEEECCCCCCCCCEEEECC     GGR
HEECCCCCHHHEHCCCEECCCCCCCCCCCCCEEEHHHCCCEEEEECCCCECCCCHHEHH     GOR
CCCCCCHHHHHHHCHHHHCCCCCCHHHHHCCCCCHHHHHHHHHHHHCCCCCCCCCCCCC     H_K
CCCCCCCHHHHHHHHHHHHHHCCHHHHHHCCCCCCCCCCCCCCCCCCCCCCCCCEECC     K_S
CCCCCCCCHHHHHHHHHCHHCCCCCCCHCCCCECHHEECEECCCCCCCCCCCCECCC     JOI
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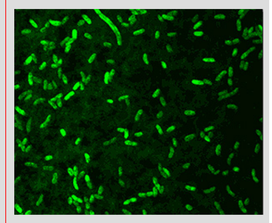
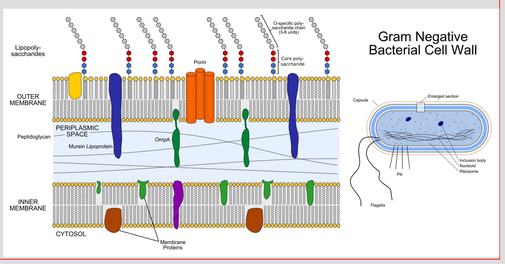
DISCUSSION

Being able to inhibit the communication among bacteria in a biofilm would decrease its overall virulence. In this study, we seek to compare the amino acid sequences, hydrophobicity, and protein structure of five Gram negative bacteria species that hold the LuxR protein.

Our goal is to contribute to how to most effectively modify furanones, natural bacteria resistance, in order to inhibit quorum sensing in biofilms. We specifically looked at five Gram negative bacteria:

Aliivibrio fischeri
Photobacterium leiognathi
Vibrio cholerae
Vibrio parahaemolyticus
Photobacterium phosphoreum





FUTURE WORK

1. Gather further data on each DNA sequence, such as the prediction of the protein's secondary structure and transmembrane segments.
2. Analyze other Gram-negative bacteria, aside from the current five explored in this study.
3. Explore current furanone variant studies.
1. Work to remove biofilms by preventing initial growth and sterilizing the colony, as opposed to solely inhibiting communication, which is the focus of this study.

REFERENCES

Hentzer, Morten, and Kathrin Riedel. "Inhibition of Quorum Sensing in Pseudomonas Aeruginosa Biofilm Bacteria by a Halogenated Furanone Compound." Department of Molecular Microbiology, 2002. Web. <http://www.researchgate.net/publication/11574813_Hentzer_M_Riedel_K_Rasmussen_TB_Heydorn_A_Andersen_JB_Parsek_MR_et_al._Inhibition_of_quorum_sensing_in_Pseudomonas_aeruginosa_biofilm_bacteria_by_a_halogenated_furanone_compound._Microbiology_148_87-102>.

Koch, B., T. Liljefors, T. Persson, J. Nielsen, S. Kjelleberg, and M. Givskov. "The LuxR Receptor: The Sites of Interaction with." Microbiology, 8 July 2005. Web. <http://mic.microbiologyresearch.org/content/journal/micro/10.1099/mic.0.27954-0?crawler=true&mimetype=application/pdf>.

Nadell, Carey D., Joao B. Xavier, Simon A. Levin, and Kevin R. Foster. "The Evolution of Quorum Sensing in Bacterial Biofilms." Public Library of Science, 28 Jan. 2009. Web. <http://journals.plos.org/plosbiology/article?id=10.1371%2Fjournal.pbio.0060014>.

Oinuma, Ken-Ichi, and Peter Greenberg. "Acyl-Homoserine Lactone Binding to and Stability of the Orphan Pseudomonas Aeruginosa Quorum-Sensing Signal Receptor QscR." *Journal of Bacteriology*. American Society for Microbiology, Jan. 2011. Web. <http://jb.asm.org/content/193/2/421.full>.

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