Killing Legionella Pneumophila Softly

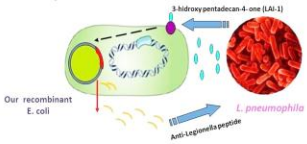
For: iGEM Competition, 2013
Medical Facility: Baskent Faculty of Medicine

Başkent Meds 2013

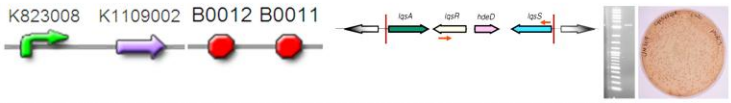
Introduction

Legionella pneumophila are gram negative pathogen bacteria. Nearly 50 *Legionella* subspecies are identified, and *L. pneumophila* is the cause of 90% of *Legionella* sourced infections. It expresses its virulence factor in alveolar cells of human lung, where it lives intracellularly. This bacteria can be also found as intracellular parasite of amoeba in water. Bacteria are present inside biofilms and residues on warm and moist surfaces, which act as a host for *Legionella* particularly when hot water systems and aerosolized usage of water are considered, *Legionella pneumophila* generates serious threat against human health. *L. pneumophila* can be found in coolers, shower heads and air conditioners of community building.

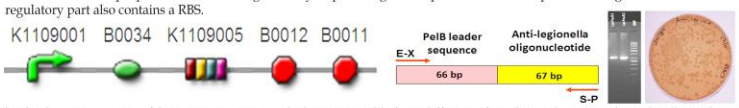
Project Overview



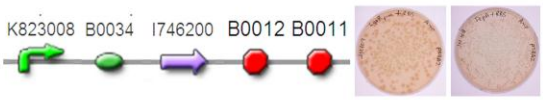
The system is biosensor *E. coli* cells which can recognize *L. pneumophila* specifically at species level and respond to that recognition by producing and secreting anti-Legionella peptide which is a peptide produced by some *Staphylococcus* strains. Recognition of population density in environment by bacteria is possible by sensation of species specific signaling molecules. With quorum sensing (QS), via gene expression; symbiosis, virulence, antibiotic production and biofilm formation can be controlled at population level. In *L. pneumophila*, lqs (legionella quorum sensing) gene locus, which is composed of lqsA (autoinducer synthase), lqsR (response regulator) and lqsS (sensor kinase) genes, is responsible for this mechanism. lqsS, which recognizes *Legionella* presence due to lqsA secretion to environment, affects cellular metabolism by phosphorylation of lqsR. Our system is composed of 3 expression "cassettes". In the first cassette; a translational lqs response unit is generated with lqsS and lqsR system elements. Source of lqs region is and pNT-1 plasmid (Cellular Microbiol 9:2903-20, 2007). The LqsR and LqsS genes have illegal restriction enzyme cutting sites inside their coding regions. So, BBa_K1109002 composite part was amplified with primers having SpeI cutting sites, and cloned into the downstream of a constitutive promoter in the pSB1C3.



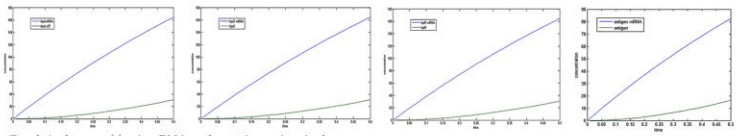
Anti-legionella (Warnericin RK) peptide induces channel formation at a low concentration, and a detergent-like solubilization of the membrane at high concentration. ORF for anti-legionella peptide specifically encodes for a 22 amino acid peptide. Anti-legionella unit is a composite Biobrick for suitable suffix and prefix regions. It contains a PelB leader sequence in the upstream of the anti-legionella peptide ORF to direct the peptide outside the cell. Source is synthetic oligonucleotides. In the second cassette; anti-legionella unit Biobrick is transcribed under lqsR promoter which is regulated by response regulator lqsR itself. Promoter part was designed as a Biobrick. The regulatory part also contains a RBS.



The third cassette contains febA L8T pore protein which is responsible from diffusion of synthesized proteins through cell membrane to outside of the cell. It is transcribed under a constitutive promoter and with RBS in the 5' region of ORF. Anti-legionella peptide, directed to periplasmic space, is secreted from cell in this manner. Source of the parts is the iGEM distribution kit.



Modelling



Graph 1: change of fepA mRNA and protein on time is shown.
Graph 2: change of lqsS mRNA and protein on time is shown.
Graph 3: change of lqsR mRNA and protein on time is shown.
Graph 4: change of antileg mRNA and protein on time is shown.

Safety

Although we use *Legionella pneumophila* genes, we work on microorganisms with biosafety risk level 1. In addition our experiments are performed inside a laminar flow cabinet to reduce the risk. We are always wearing lab coats, gloves and googles while working in the lab.

We cloned autoinducer synthase gene of *L. pneumophila* to *E. coli*, and we produced *L. pneumophila* mimicking *E.coli*. So we aim to observe the response our processed QS switch by using the transformed *E.coli* producing autoinducer of *Legionella* instead of the *L.pneumophila* cell itself.

Conclusion

In conclusion, detection and prevention of colonization of *Legionella pneumophila* are substantially costly and requires long processes. Our biological system can both provide detection and prevent colonization that occurred via high bacterial load with a considerably low cost. Since is works with natural biological expression process, the system can react to environmental alterations in a small amount of time. As the system can settle itself on colonization surfaces, it is easy to use after the first inoculation. The system does not constitute any risks in means of biosafety because *Escherichia coli* strains which are noninfectious, present in environment and has low virulence are manipulated and developed.

Human Practice

♥ 2012 and 2013 Baskent University Medical School Symposium

♥ Presentation for ITU İgem Team's organization

Our YouTube Channel

♥ Presentation for IDF Bilkent High School to introduce iGEM High School Competition

Time Line

Med School

2010
First year as a med student.

August 2011
The idea of participating in iGEM.

September 2011
New semester at med school.

October 2011
Learning anatomy at school.

November 2011
Winter time. Our city is getting colder, our exams are getting harder.

December 2011
Studying physiology.

January 2012
Happy new year!

February 2012
Semester break after all the hard work and rough exams!

March 2012
School and exams.

April 2012
Med school is getting harder at every step.

May 2012
Our first presentation about our iGEM project in our school.

June 2012
School year is finished with hardest final exams ever!

July 2012
Summertime sadness. Thank God, there are no exams.

August 2012
Holiday is almost over.

September 2012
Just started 3th grade in med school. We're really excited.

October 2012
Pathology, pharmacology, microbiology...

November 2012
Making so many things at the same time, exams...

December 2012
We waited for apocalypse...

January 2013
Happy new year, again!

February 2013
Winter is cold,
Exams are not that much.

March 2013
I see dead people! (cadavers)

April 2013
Trying to learn all the drugs in the world for med school.

May 2013
2nd presentation at school with METU Team this time.

June 2013
3th year at Med School is over.

July 2013
Holiday.

August 2013
Holiday.

September 2013
Now we are resident doctors!

October 2013
Medical internships had just begun!

iGEM

September 2011
Learning how to use lab equipments.

October 2011
Working on genetics and biotechnology at lab.

November 2011
Brainstorming to find a cool project.

December 2011
Couldn't find an idea yet, we keep thinking and thinking while making some experiments for practicing.

January 2012
We found our idea for the project. New year is a charm.

February 2012
First semester break with iGEM, we're in lab, no time for partying.

March 2012
Learning how to design an iGEM project.
Practicing in lab almost every single day.

April 2012
Finally figured out how to use biobricks for our project.

May 2012
Still working for experiments.

June 2012
This will be our first summer together in lab for iGEM.

July 2012
Spending our summer holiday in lab. Experimenting to the end of time. We're worrying if we could finish all the work for participating in 2012 Europe Jamboree.

August 2012
We'll withdraw this year and finish our work for iGEM 2013.

September 2012
Going lab after school time.

October 2012
Working in lab in evenings.

November 2012
and experiments.

December 2012
while waiting for PCR, they both didn't happen.

January 2013
Still experimenting.

February 2013
but our lab is very warm.

March 2013
Experiments are going fine, finally thinking about human practice ideas.

April 2013
T-shirt designing, non-stop experimenting.

May 2013
We're making progress.

June 2013
Last summer in lab begins.

July 2013
Hot summer nights with last experiments.

August 2013
Human practice. Shooting great videos with great people out there in Ankara.

September 2013
Presentation for high school iGEMers in Ankara IDF Bilkent High School. We sent our parts, getting ready for Lyon.

October 2013
Managed to make our wiki beautiful before wiki freeze, now it's time to pack our bags for Lyon!

"Our true mentor in life is science."

Our Sponsors

TUBİTAK
ATA DIAGNOSTICS
Agilent Turkey

This study was approved by Baskent University Institutional Review Board (Projects No: DA12/05 and DA13/06), and supported by Baskent University Research Fund.

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