MISSION:

IMPOSSIBLE
Have you ever.....
Have you ever.....
“many problems are caused by bacteria...”
“especially when they are forming...”
Why Biofilm is Dangerous

? Dose of Antibiotic
Why Biofilm is Dangerous

1. Dose of Antibiotic
Why Biofilm is Dangerous

1. Dose of Antibiotic
Why Biofilm is Dangerous

500 Dose of Antibiotic
Why Biofilm is Dangerous

500 Dose of Antibiotic
Bacteria forming Biofilm
Bacteria forming Biofilm

- Vibrio cholerae
- Staphylococcus aureus
- Pseudomonas aeruginosa
- Bacillus subtilis
- Klebsiella pneumoniae
Bacteria forming Biofilm

*Vibrio cholerae*
Bacteria forming Biofilm

*Vibrio cholerae*
Vibrio cholerae

CHOLERA

Number of Cholera Cases Reported (1989-2013)

WHO, 2014
Vibrio cholerae

CHOLERA
Vibrio cholerae

CHOLERA

Sanitation

Neglection

Unreported Cases
CHOLERA
20.49% population is at risk
5 Million people infected annually
100-120 thousands death

WHO, 2013
**Vibrio cholerae – Life Cycle**

1. **Colonization & multiplication in intestinal epithelium**
2. **Released into the environment by diarrhea**
3. **BIOFILM**
4. **Persistence in aquatic reservoirs**
5. **Passes through the gastric acid**
6. **Enters into the human body through oral**

Adapted from Shao Yi, 2014
“Our Genius *E. coli* can solve this problem...”

*How?*
Project Overview

Hunting → Biofilm Degrading → Killing

Mr. GE
Sensing

*V. cholerae*'s presence
The Genius *E. coli*’s Abilities

**Motility**
Moving towards *V. cholerae*
The Genius *E. coli*’s Abilities

DEGRADING BIOFILM

- Alpha amylase
- Nuclease
The Genius *E. coli*'s Abilities

Peptide 1018

KILLING
The Genius *E. coli*’s Abilities

KILLING

Peptide 1018
Sensing with CqsS Receptor

CAI-1
(S)-3-hydroxytridecan-4-one
1. Indigenous in *V. cholerae*
2. Main quorum-sensing signal
3. Detected by CqsS system

The detection of CAI-1 triggers the cascading autophosphorilation and activates the virulence protein regulator, HapR.
Sensing with CqsS Receptor
Sensing with CqsS Receptor

- CqsS receptor
- CAI-1
- YbeL → CqsS → YaiN
- V. Cholerae (biofilm)
Overexpressing CqsS

Using **YbeL** – **YaiN** fusion protein system

- uncharacterized protein in *E. coli*
- a formaldehyde operon protein regulator in *E. coli*
Overexpressing CqsS

Using \textbf{YbeL} \textbf{-} \textbf{YaiN} fusion protein system

Used to overexpress transmembrane protein, because transmembrane protein is usually insoluble and expressed in inclusion body.
Motility with Activated CheZ

CheZ is a gene responsible for *E. coli* to ‘run’ forward.
Biofilm Degrading Enzyme

Nuclease

Alpha amylase
Biofilm Degrading Enzyme

- **Nuclease**
  - coded by *Nuc* gene
  - cut phosphodiester bond
  - indigenous from *Staphylococcus aureus*

- **Alpha amylase**
  - coded by *MaIS* (periplasmic α-amylase) gene
  - hydrolysis α-1,4-glicosidic bond
  - indigenous from *E. coli*
Biofilm Degrading Enzyme

Hunting

Biofilm Degrading

Killing

Nuclease

Alpha amylase

MaIS
Biofilm Degrading Enzyme

Why HlyA?

Nuclease + HlyA

Alpha amylase + HlyA

Hunting

Biofilm Degrading

Killing
+ HlyA Secretion Tag

without HlyA

with HlyA

Nuc
MalS
HlyA
+ HlyA Secretion Tag

without HlyA

with HlyA

Hunting

Biofilm Degrading

Killing
Improvement of HlyA Tag

From UNICAMP EMSE Brazil IGem Team

2009

A

\text{NNNNNNN} \text{TAATAA} \text{TACTAGAT} \text{TTAGCCTAT}

\begin{itemize}
  \item gene to be fused with HlyA
  \item stop codon
  \item 3A scar (7 bp)
  \item HlyA
\end{itemize}

B

\text{NNNNNNN} \text{TAATAA} \text{TACTAGAT} \text{TTAGCCTAT}

\begin{itemize}
  \item gene to be fused with HlyA
  \item stop codon
  \item 3A scar (7 bp)
  \item HlyA
\end{itemize}

\text{[triplets reading frame]}

C

\text{NNNNNNN} \text{TACTAGAT} \text{TTAGCCTAT}

\begin{itemize}
  \item gene to be fused with HlyA
  \item 3A scar (for ATG part)
  \item methionine
  \item HlyA
\end{itemize}

\text{[triplets reading frame]}
Starch Hydrolysis Agar Assay

1. Centrifuge LB broth containing the engineered E. Coli
2. Separate supernatant and pellet phase
3. Pipette the supernatant and pour into the starch agar
4. Incubate the starch agar and flood by iodine

Observe the clear zone

Plaque or clear zone (positive result)

Dark zone (negative result)
Starch Hydrolysis Agar Assay

Notes:
- MalS-HlyA is denoted alpha-amylase with secretion tag
- Plasmid only as negative control, no expression
- WT is wild type E.coli top10, no plasmid
Starch Hydrolysis Agar Assay

RESULT

MaLS-HlyA works as expected

Notes:
MaLS-HlyA is denoted alpha-amylase with secretion tag
Plasmid only as negative control, no expression
WT is wild type E.coli top10, no plasmid
Biofilm Assay

++18 Hours

Incubate LB Broth containing sample bacteria overnight

++48 Hours

Pipette the incubated LB Broth and add into the micro titer plate then incubate for 48 hours in 37°C

+++5 Hours

Take out the incubated LB broth. Now, only biofilm remains.

Add 200uL fresh LB broth into the well which contains biofilm

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Add 2uL “Genius E.coli” into the well containing fresh LB Broth and incubate it for 5 hours

Remove all of the LB from the well, sparing the biofilm.

Add crystal violet, which will colorize the biofilm.

Read the absorbance under 595 nm

Hunting

Biofilm Degrading

Killing
Observe the Blue Color Gradation

Biofilm was not formed

Thin biofilm

Thick biofilm
Biofilm Assay

RESULT (a)

- Negative control
- *Vibrio cholerae*
- *Bacillus subtilis*
- *Pseudomonas aeruginosa*
- *Staphylococcus aureus*
- *Klebsiella pneumoniae*
- *Escherichia coli*
- Blank
Biofilm Assay

RESULT (b)

Negative control
- *Vibrio cholerae*
- *Bacillus subtilis*
- *Pseudomonas aeruginosa*
- *Staphylococcus aureus*
- *Klebsiella pneumoniae*
- *Escherichia coli*
- Blank

- Nuc-HlyA
- Mix
- No Treatment

1 2 3 4 5 6 7 8 9 10 11 12
Average Reduction of Biofilm in Several Bacteria (%)
Biofilm degrading device works as expected.
Peptide 1018

- Broad spectrum anti-biofilm substance
- Identified as innate defense regulator
- Blocking (p)ppGp, stress response 2nd messenger, preventing biofilm formation and killing the cells
Peptide 1018

Safety: the peptide will kill both of them!

- Broad spectrum anti-biofilm substance
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Peptide 1018

Safety: the peptide will kill both of them!

- Broad spectrum anti-biofilm substance
- Identified as innate defense regulator
- Blocking (p)ppGp, stress response 2\textsuperscript{nd} messenger, preventing biofilm formation and killing the cells

Peptide 1018 + H\textsubscript{ly}A
Peptide 1018 – Toxicity Assay

The reduction of cells density is occurred when Peptide 1018 is expressed.

The increase of cells density is occurred when Peptide 1018 is not expressed.
Peptide 1018 – Toxicity Assay

Killing Module of Peptide 1018 works as expected

- The reduction of cells density is occurred when Peptide 1018 is expressed
- The increase of cells density is occurred when Peptide 1018 is not expressed
BioBricks Confirmation

EP: EcoRI + PstI digestion
Our project of "Genius E. coli" could be an effective solution to solve biofilm problem
Human Practices

SynBio Shout Out!

Expert Talking

Prof. Masafumi Yohda
Biotechnology and Life Science
(Tokyo University of Agriculture and Technology)

Aroem Naroeni, DEA, Ph. D
Biosafety Officer
IHV/CH-UI

“How do movies influence people?”
# Team Achievements

## Hunting

1. Sensing New Part: CqsS (reversed histidine kinase quorum sensing system from *Vibrio cholerae*; Bba_K1344010)
2. Sensing New Parts: YbeL (Bba_K1344011) and YaiN (Bba_K1344012)
3. Motility Device: CheZ-GFP

## Biofilm Degradation

1. Device: J23100-(RBS-MaIS)-HlyA and J23100-RBS Nuc-HlyA
2. Device: (RBS-MaIS)-HlyA and (Nuc-HlyA)
3. Improving HlyA Parts from UNICAMP EMSE Brazil
4. Doing the characterization successfully

## Human Practice

1. SynBio Shout Out!
2. Interviewing Experts
3. Movie

## Collaboration

1. Helping Tokyo-NoKoGen Team and having collaboration with the team in human practice
2. Helping Paris Bettencourt Team with their MOOC Project

## Killing

1. New Part: Peptide 1018
2. Device: T5-RBS-Peptide 1018
3. Improvement Parts: Thermo regulated promoter-Peptide 1018
4. Doing the characterization with positive results
• **Fuente-Núñez, et al.** for the discovery and characterization of peptide 1018, a substance that specifically kill biofilm-making bacteria. The discovery of peptide 1018 has allowed us to design a synthetic bacteria which kills biofilm-making pathogen but harmless to native, planktonic bacteria.

• **UNICAMP EMSE Brazil iGEM Team 2009**, for the development and characterization of Type I secretion system in *E. coli*. We used the *E. coli* type I secretion system to export biofilm degrading enzyme to extracellular environment.

• **Mr. Budiman Bela**, for his insight on pathogen behaviour and stress response that helps us to decide which quorum sensing mechanism works best for our purposes.

• **Muhammad Hanifi**, for his insight on the structure and composition of bacterial biofilm and thus, leading to the right substance to degrade them.
Team Profile

Team Members

Siska  Anggoro  Etri  Yuda  Diana  Vanessa  Robby  Anasthasia

Advisors

drh. Yulianty, M. Biomed  Nada Fitriah, S. Si, M. Biomed  M. Hanifi  Wian  Taufik  Teguh  Danny

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Supported By
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