- Target and kill antibiotic resistant pathogenic bacteria and cancer cells
- Implementing a killer-prey-system with two different *E.coli* strains
Overview

Sensing

Phage Killing

Colicin Killing
Prey cells produce LuxS secretion
Chimeric receptor triggers chemotaxis → AI-2 secretion

Sensing System
Overview
Sensing System
LuxQ-Tar Fusion Protein

General structure of LuxQ and Tar

Construct 1: TM2 from LuxQ
Construct 2: TM2 from Tar

Legends:
TM: transmembrane domain  PD: periplasmic domain  CD: cytoplasmic Domain

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Results
Expression Test with Fusion-YFP constructs

LuxQ-Tar fusion protein is expressed and located in the membrane
Killing System I – Killing by Phages

Overview

λ repressor
conjugative proteins
λ-phage genome

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Phage-Killing Modeling

Model overview

\[
\frac{dx}{dt} = \alpha x \left(1 - \frac{x(t) + y(t) + u(t) + z(t)}{\kappa}\right) x(t) + \beta v(t)x(t) - \lambda u x(t)
\]

\[
\frac{dy}{dt} = \mu(t)x(t) + \kappa z(t) + \beta v(t - \Delta) x(t - \Delta) - \lambda v y(t) - \beta v(t)x(t)
\]

\[
\frac{dz}{dt} = L(\beta v(t - \Delta) x(t - \Delta) + \kappa z(t - \Delta)) - \mu v y(t) + \beta v(t)x(t)
\]

\[
\frac{dv}{dt} = \alpha u \left(1 - \frac{x(t) + y(t) + u(t) + z(t)}{\kappa}\right) u(t) - \lambda u(v) - \kappa z(t)
\]

\[
\frac{dz}{dt} = \mu v x(t) - \kappa z(t)
\]
High initial killer cell density is not necessary for efficiency of the system: 10 killer cells are able to kill $10^9$ prey cells \textit{in silico}
Killing System I – Killing by Phages

Cloning strategy

PCR amplification with CmR specific primers from the λ- genome

Engineering of the λ-phage succesful
Conjugation tests with engineered λ- phages

Numerical values for conjugation rate obtained for modeling

Conjugation Kinetics

conjugation rate: $1.05 \times 10^{-12}$ [ml • min$^{-1}$ • cell$^{-1}$]

System is functional
Killing System II – Killing by Colicins

Overview

Colicin AI-1
LuxR
LuxI
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Prey produces LuxI, killer produces LuxR. LuxI produces Al-1 which diffuse through the medium. LuxR-Al-1 complex activates colicin E1 production. Killer cell lyses and releases colicins.

Colicin kills prey cell.

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Characterization: Determination of AI-1 concentration in culture supernatants

Maximum \( c(\text{AI-1}) \approx 1250 \text{ pM} \)
Characterization: Determination of killing efficiency on different induction levels and prey-killer ratios

Lysis test - Killer

Characterization: Determination of killer cell lysis for colicin release

Lysis effect observed for $c(AI-1) \geq 5$ nM

Lysis effect observed for $c(AI-1) > 500$ pM

...prey-killer ratios up to 100:1
Killer cells prepared in different ratios with AI-1 producing sender cells.

- Growth curve determination of sender cell population.
- Complete extermination of prey population.
- Sufficient AI-1 production by the sender cells for killing induction in ratios up to killer:prey 1:25

**System Test**

**Sender cells induce colicin production by killer cells**

- **t = 0 min**
- **t = 30 min**
- **t = 60 min**

**Killer-Prey Test**

- 5.5 × 10^4
- 5

**Constructive Killer-Prey-System is functional with high efficiency**

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Cancer Cell Test

Toxic Effect on eukaryotic cancer cells

- Cancer cells (MCF-7) treated with colicin E1 producing killer cells
- Propidium iodide (PI) used to detect dying cells
- Percentage of PI stained cancer cells, thus dying ones were measured over time
  → Colicin E1 producing killer cells are able to kill eukaryotic cancer cells
  → Approach for medical treatment

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Sensing Colicin-Killing Modeling

Model overview

AI-2 acts as chemoattractant for the killer cells. They can sense the prey strain and swim towards them by using the AI-2 signal gradient.

Studied spatio-temporal behavior of predator-prey system using Partial Differential Equations (PDEs).

Model equations: (PDEs)
Sensing-Colicin-Killing Modeling

Simulation Results

low chemotactic activity

high chemotactic activity
Sensing

Colicin Killing

Human Practice - Science Communication

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Summary

- Creation of Al-2 Sender Part
- Creation of chemotaxis LuxQ-Tar fusion receptor
- Expressed and located in Membrane

- Cloning and Characterization of bacteriophage λ cl
- Assembly of modified bacteriophage λ
- Conjugation of modified bacteriophage λ

- Creation of Al-1 Sender Part
- Creation of Al-1 inducible colicin producing killer part

- Verification of the colicin prey-killer system
- Killer activity on eukaryotic cancer cells
At the end of the day...
Acknowledgements

We thank all our supporters
At the end of the day…