E. teamwork

“Engineering a synthetic microbial consortium“

TECHNISCHE UNIVERSITÄT BRAUNSCHWEIG

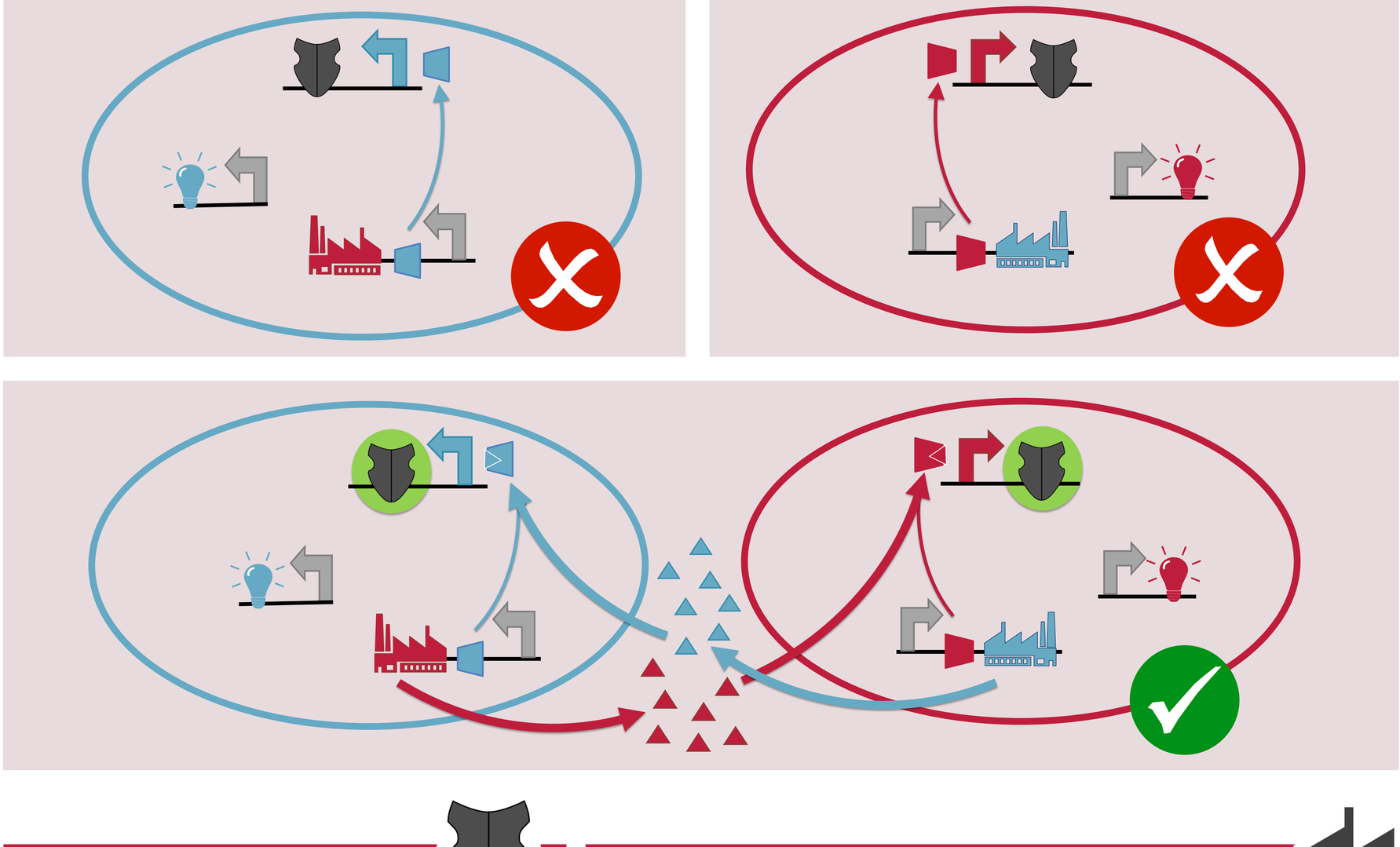
Abstract

Microbial consortia are common in nature and offer a broad variety of new applications in synthetic biology due to their ability to perform more complicated tasks than monocultures.
From wastewater treatment to the assistance in food digestion and synthesis of complex biological compounds, engineering microbial consortia facilitates processes of great importance to humans [1]. When using genetically engineered microbes in a mixed culture, one of the members could become dominant. An unbalanced consortium may have unfavorable effects on the whole biotechnological process [2]. Here we constructed a model system for a self-regulating synthetic microbial consortium of two *Escherichia coli* strains based on a cross-inducible antibiotic resistance. We engineered genetic devices to enable the growth of two different *E. coli* strains in a consortium via exploiting components of the quorum sensing systems las and rhl of *Pseudomonas aeruginosa*.

Human Practices

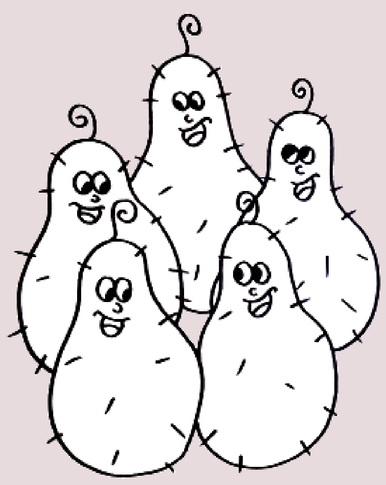
How to succeed in Public Relations“? Our guide helps future iGEM Teams to tune the outreach of their Human Practices activities.

The *E. teamwork* System



Quorum Sensing

Bacteria can coordinate community-wide behavior by the exchange of small molecules (homoserine lactones) within or between single populations.
Various cell density dependent events like biofilm formation [3] or the expression of virulence factors [4] are dependent on this phenomenon called quorum sensing (QS).
Having an immediate regulatory effect on transcription level signal molecules of QS systems can be exploited in synthetic biology.

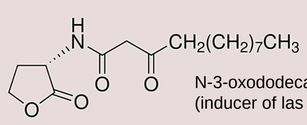


Operation Unit 1 – Inducible Ampicillin Resistance

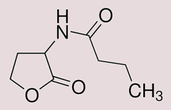
- Expression of beta-lactamase is induced by an active complex of transcription factor LasR or RhlR and the corresponding inducer

Operation Unit 2 - Induction Complex Production

- Constitutive expression of corresponding inactive transcription factor (*lasR or rhlR*) and acyl homoserine lactone synthase (*lasI* or *rhlI*) in two different cells creating dependency between them
- Synthesis of specific inducer molecules which are secreted into the medium and taken up by the other cell



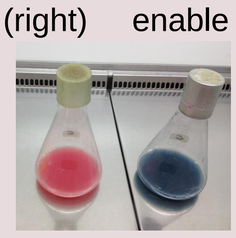
N-3-oxododecanoyl homoserine lactone (inducer of las system)



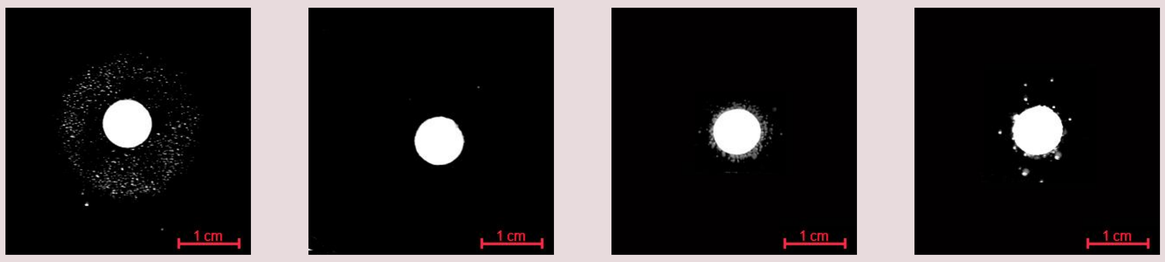
N-butyryl homoserine lactone (inducer of rhl system)

Operation Unit 3 Reporter

- Constitutive expression of the chromoproteins eforRed (left) and aeBlue (right) enable distinction of the two strains with naked eye



Cross Induction



Positive control (medium + synthetic inducer)

Negative control (inducer free medium)

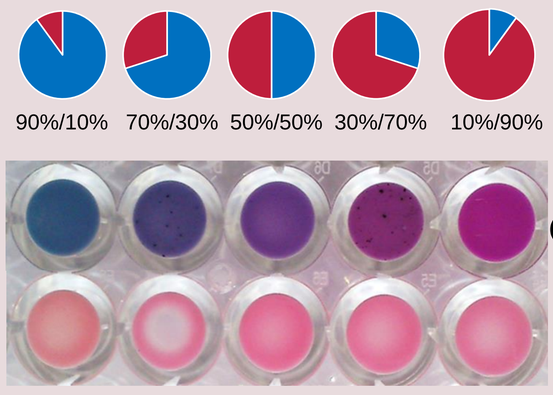
Construct 1 + supernatant of construct 2

Construct 2 + supernatant of construct 1

Cross induction of strains

Cell growth on ampicillin supplemented agar plates can be induced by the complementing strain of the consortium. A piece of filter paper was soaked with sterile supernatant of the complementing strain and placed on top of ampicillin supplemented agar dishes with plated bacteria.

Regulation in Mixed Cultures

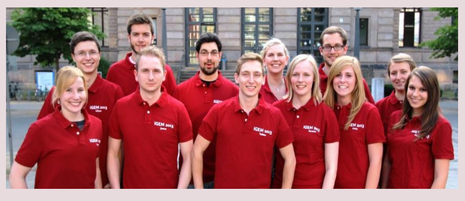


The regulatory devices affect the composition of mixed cultures.
After inoculation with different ratios of two strains bearing the regulatory devices the composition remained different in chloramphenicol containing medium (top, unregulated) but was found to be very similar in ampicillin containing medium (bottom, regulated).

Sponsors

Promega, dbt, Technische Universität Braunschweig, YUMAB, Braunschweigischer Hochschulbund e.V., BÜRGERSTIFTUNG Braunschweig, JRS PROZESSTECHNIK GMBH & CO. KG WERK CALENBERG, ASPHALTTECHNIK LOHNDIENSTLEISTUNGEN, Ecki Wohlgehagen-Stiftung

Team



References

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2. 2. Shong, J., M.R. Jimenez Diaz, and C.H. Collins, *Towards synthetic microbial consortia for bioprocessing.* Curr Opin Biotechnol, 2012. **23**(5): p. 798-802.
3. Greenberg, E.P., *Bacterial communication and group behavior.* J Clin Invest, 2003. **112**(9): p. 1288-90
4. De Kievit, T.R., et al., *Quorum-sensing genes in Pseudomonas aeruginosa biofilms: their role and expression patterns.* Appl Environ Microbiol, 2001. **67**(4): p. 1865-3.