Combinatorial Genetic Approach to Dissect the Mechanisms of Biofilm-Associated Infection

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IMPACT ON AMR
In the era of antimicrobial resistance, bacterial biofilms serve as incubators for dissemination of resistant traits, at the same time shielding bacteria from antibiotics. Minimal inhibitory concentrations of antibiotics for bacteria within biofilms are typically much higher than for planktonic cells, making biofilm-associated infections not only much harder to clear, but contributing to a tendency to relapse. Therefore, multifaceted strategies should be deployed to eradicate biofilm-associated infections.

Entercoccal infections are often biofilm-associated, hard to treat, and may become life-threatening. Despite the importance of biofilms to enterococcal infection, analysis of biofilm factors has been mono-factorial and largely in vitro.

OUR APPROACH
Utilize combinatorial genetic en mass (combiGEM)-based DNA assembly with CRISPR interference technology to identify two-component system(s) (TCS) from Enterococcus faecalis important for biofilm formation.

Test model
Nisin-inducible dual-vector CRISPRi in E. coli 1-ΔCas9 (GFP-marked OGI35R with catalytically inactive Cas9)

RESULTS
CRISPR in Enterococcus faecalis:
- Most efficient on presensitized cultures
- Efficient in:
  - global targeting
  - whole-organism silencing
  - template/non-template strand targeting
- Mimics gene knockout phenotypes in planktonic and biofilm assays
- Can be multiplexed

FUTURE VISION
- Combinatorial library design and screen for TCS genes involved in biofilm formation
- Validation of top hits in relative biofilm-associated infection models
- Novel drug-combination discovery: small molecule inhibitors screening, drug screening
- Effective combinational therapies
- Collaborative effort for drug delivery to biofilms

Platform for rapid identification of genetic combinations responsible for biofilm formation, infection and immune suppression that may serve as potent antimicrobial targets.