ARA ANTIMICROBIAL RESISTANCE Interdisciplinary Research Group

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.

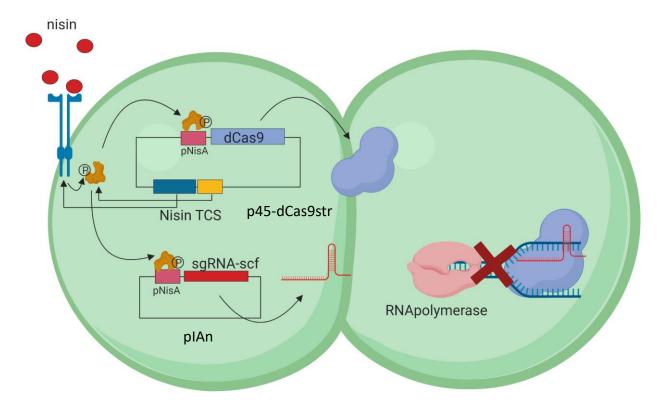
Enterococcal infections are often biofilm-associated, hard to treat, and may become life-threatening. Despite the importance of biofilms to Enterococcal infection, analysis of biofilm factors has been mono-factorial and largely in vitro.

OUR APPROACH KEY FINDINGS CombiGEM-CRISPRi to characterize TCS involved Efficient and inducible CRISPRi in Enterococcus faecalis in biofilm formation D WT_{gfp g1 (++)} WTgfp WT psgRNA-gfp p1 psgRNA-gfp g1 psgRNA-gfp g2 CombiGEM **CRISPRi** -35 Library of barcoded transcription silencing **GFP** promoter dual guide-RNA Lentiviral vector psgRNA-gfp_g3_t 18 hours 2.5 hours Β NT strand 1:30 T strand WTgfp g1(-) WT_{gfp g1 (+)} uninduced 🛨 WT pp +-🔫 p1 GC 35 • g1 GC 25 ⊦ induced **-E-** g2 GC 20 DNA – Hoechst (all cells); GFP - green Iwo-Component Regulatory System uninduce 📥 g3 GC 45 Two-component transduction to sense, ++ re-induced enable systems bacteria respond, and adapt to a wide range of re-induced induced uninduced environments, stressors, and growth induced conditions (A) Schematic diagram of gfp operon indicates 4 sgRNAs that target promoter region (gfp-p1) and gene-coding region gfp-g1, gfp-g2 and gfp-g3. Arrows indicate the distance from the start codon to the protospacer adjacent motif (PAM) that is recognized by dCas9 bound to sgRNA-scaffold. Combinational knockdown (B) 4 sgRNAs were tested for *gfp* repression activity by growing cells with or without nisin for 2.5 hours after overnight subculture. Cells were normalized, washed and analysed on flow cytometer. % of GFP expressing cells was determined by built-in Attune NxT Flow Cytometer software from 500 000 events. (C) Confocal images of 24 hours biofilms of WT, WT_{gfp} and WT_{gfp} gfp-g1 grown in plastic chambers induced (+), re-induced (++) and uninduced (-) with nisin. Cells were fixed and DNA was stained with Hoechst dye. **CRISPRi recapitulates biofilm-related knockout** Multiplex targeting

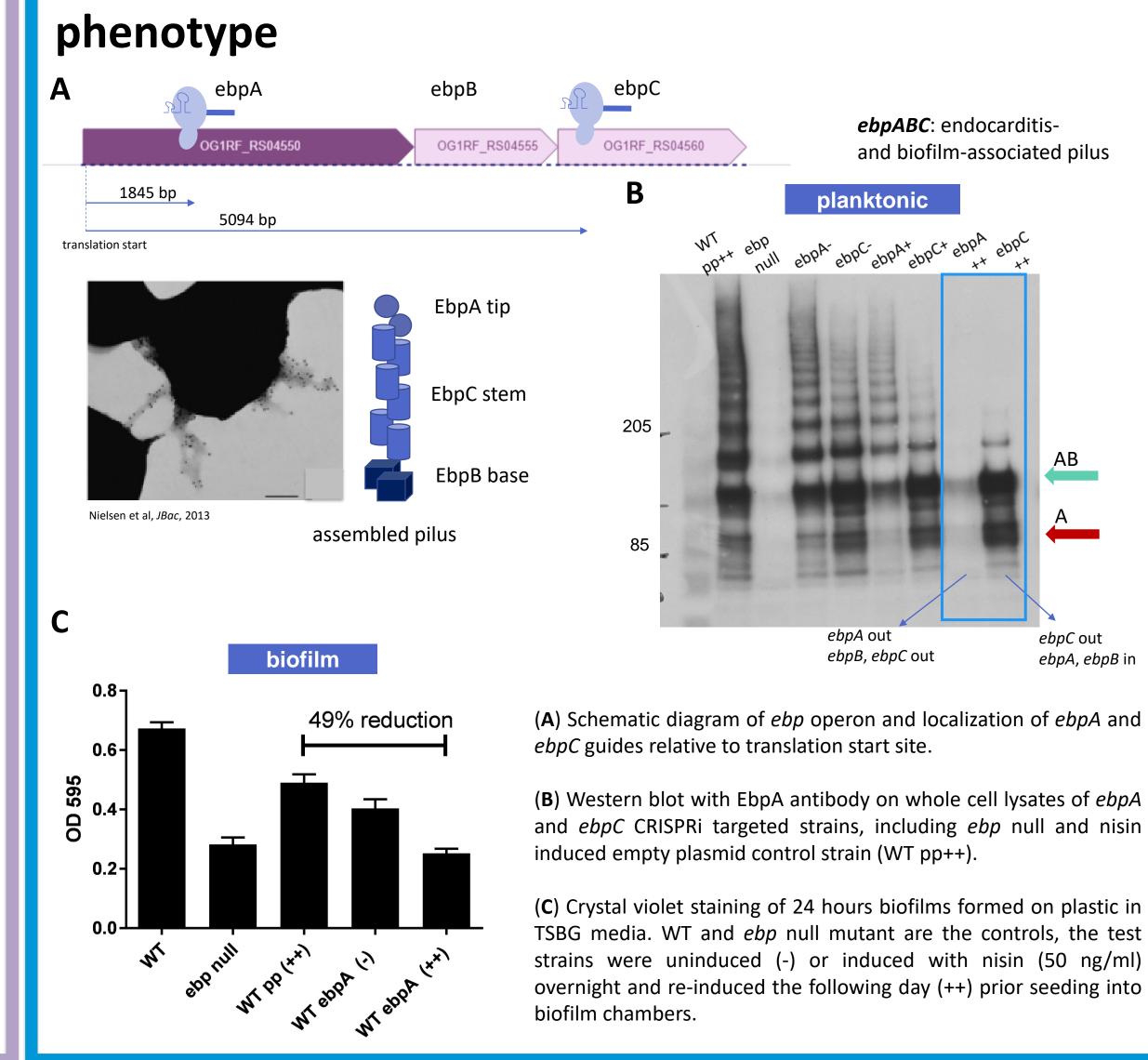
Utilize combinatorial genetic en mass (combiGEM)-based DNA assembly with CRISPR technology to identify twointerference component system(s) (TCS) from *Enterococcus faecalis* important for biofilm formation.

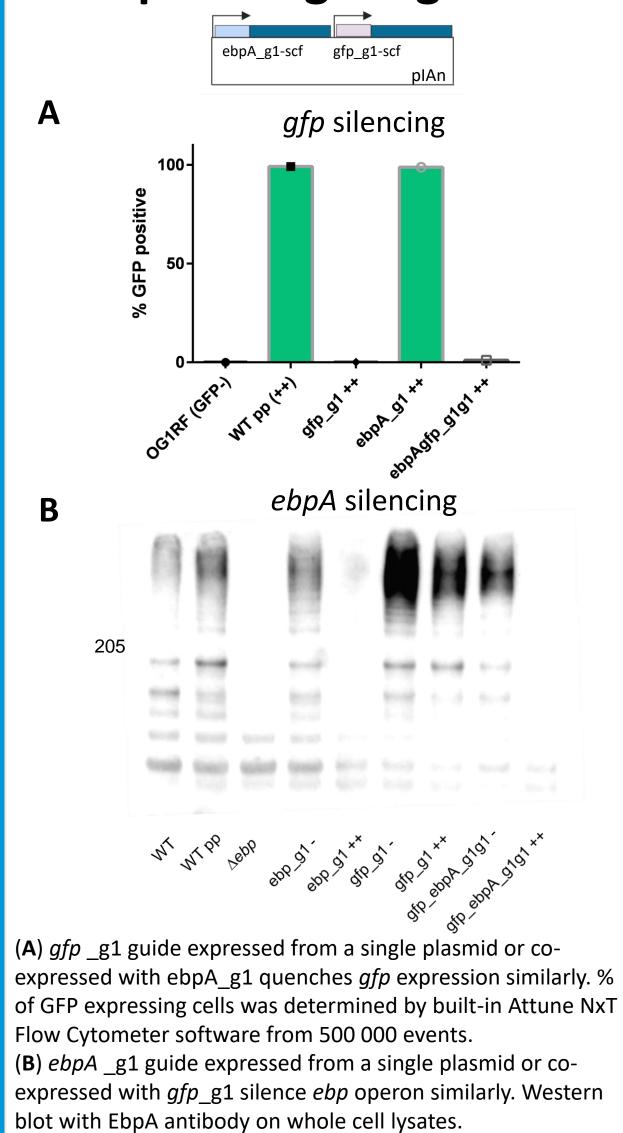
Test model

Nisin-inducible dual-vector CRISPRi in SD234::dCas9 (GFP-marked OG1RF with catalytically inactive Cas9)



Schematic diagram of CRISPRi system in Enteroccoccus faecalis dCas9 Nisin-inducible (from Streptococcus pyogenes) expressed from p45-dCas9str; nisin-inducible sgRNA (20 nt) linked to Cas9 scaffold expressed from pIAn (3.2 kb plasmid modifiable through CombiGEM).





RESULTS

FUTURE VISION

CRISPRi in Enterococcus faecalis:

- Most efficient on presensitized cultures
- Efficient in:
 - distal targeting
 - whole-operon silencing
 - template/non-template strand targeting
- Mimics gene knockout phenotypes in planktonic and biofilm assays
- Can be multiplexed

- Combinatorial library design and screen for TCS genes involved in biofilm formation
- Validation of top hits in relative biofilm-associated infection models \bullet
- Novel drug-combination discovery: small molecule inhibitors screening, drug screening

ebpC out

ebpA, ebpB in

- 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.

A translational research and entrepreneurship program that tackles the growing threat of antimicrobial resistance



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