Investigating Prophage (φSa2, φSa3) & PICI (SaPI5) Induction Dynamics in CA-MRSA Strain USA300

Via Mitomycin C induced SOS activation in reporter systems

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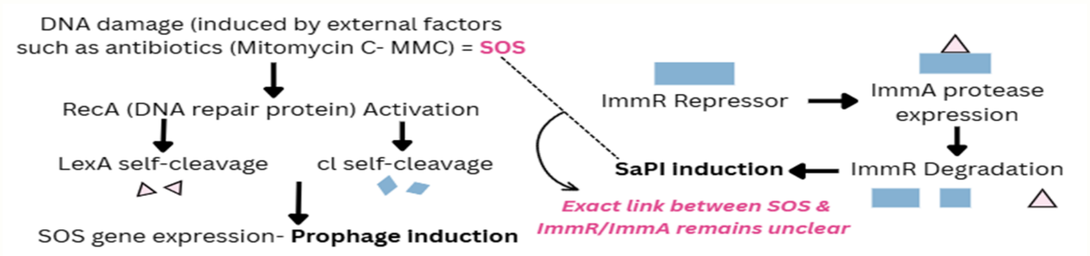
INTRODUCTION

- *Staphylococcus aureus* caused **>1.1 million deaths** in 2019[1].
- **Community-associated methicillin-resistant *S. aureus* (CA-MRSA)** is a major pathogen, resistant to a key antibiotic- methicillin.
- Mobile genetic elements (MGEs)—prophages & phage-inducible chromosomal islands (PICIs) (staphylococcal pathogenicity islands SaPI) carry **virulence & pathogenicity factors**, drive **horizontal gene transfer,** occasionally mobilizing antimicrobial resistance (AMR) genes. In USA300, key MGEs include:
  - **SaPI5** – superantigenic toxins, mobilized by helper phages.
  - **ϕSa2** – toxin-encoding prophage causing severe infection.
  - **ϕSa3** – prophage carrying immune evasion genes.
- How they synergize or compete with one another is unknown

Prophage & SaPI Induction mechanism- The SOS response[2]



AIMS

Hypothesis: MGEs are regulated differently in lab (RN4220) versus clinical strains (USA300) of *S. aureus*.

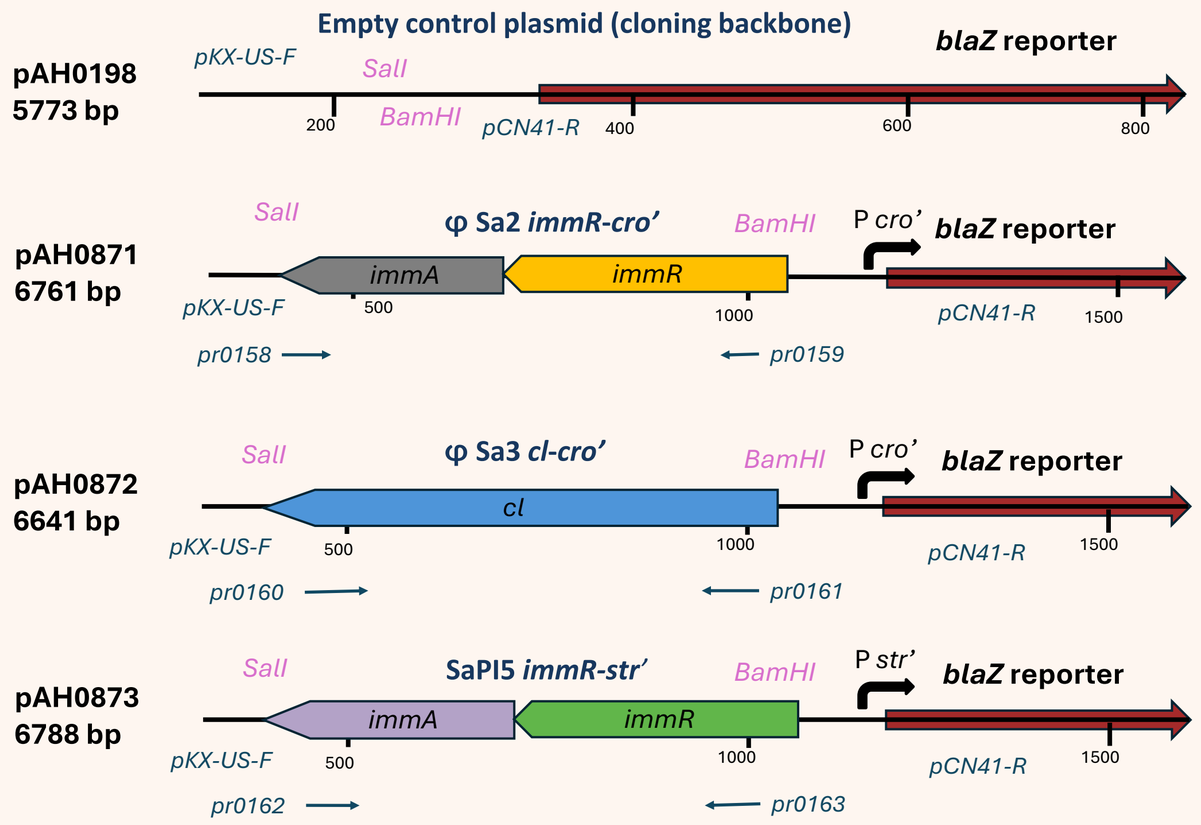
- Construct plasmid-based blaZ promoter systems
- Induce and assess promoter activity in
  1. *S. aureus* RN4220
  2. *S. aureus* USA300

METHODOLOGY

Promoter–reporter plasmids were constructed and promoter activity measured by colour change assay: β-lactamase (*blaZ*) hydrolyzes nitrocefin, turning it yellow to red.

1. Inoculation and extraction of empty vector plasmid
2. Promoter amplification via PCR
3. *Sal*I/*Bam*HI digest, ligate inserts
4. *E. coli* IM01B transformation
5. Sequencing
6. Electroporation into RN4220
7. USA300: ϕ11 transduction
8. Assays (± MMC (2 µg/mL); 0–120 min; OD486/OD600)

Plasmid Constructs



RESULTS

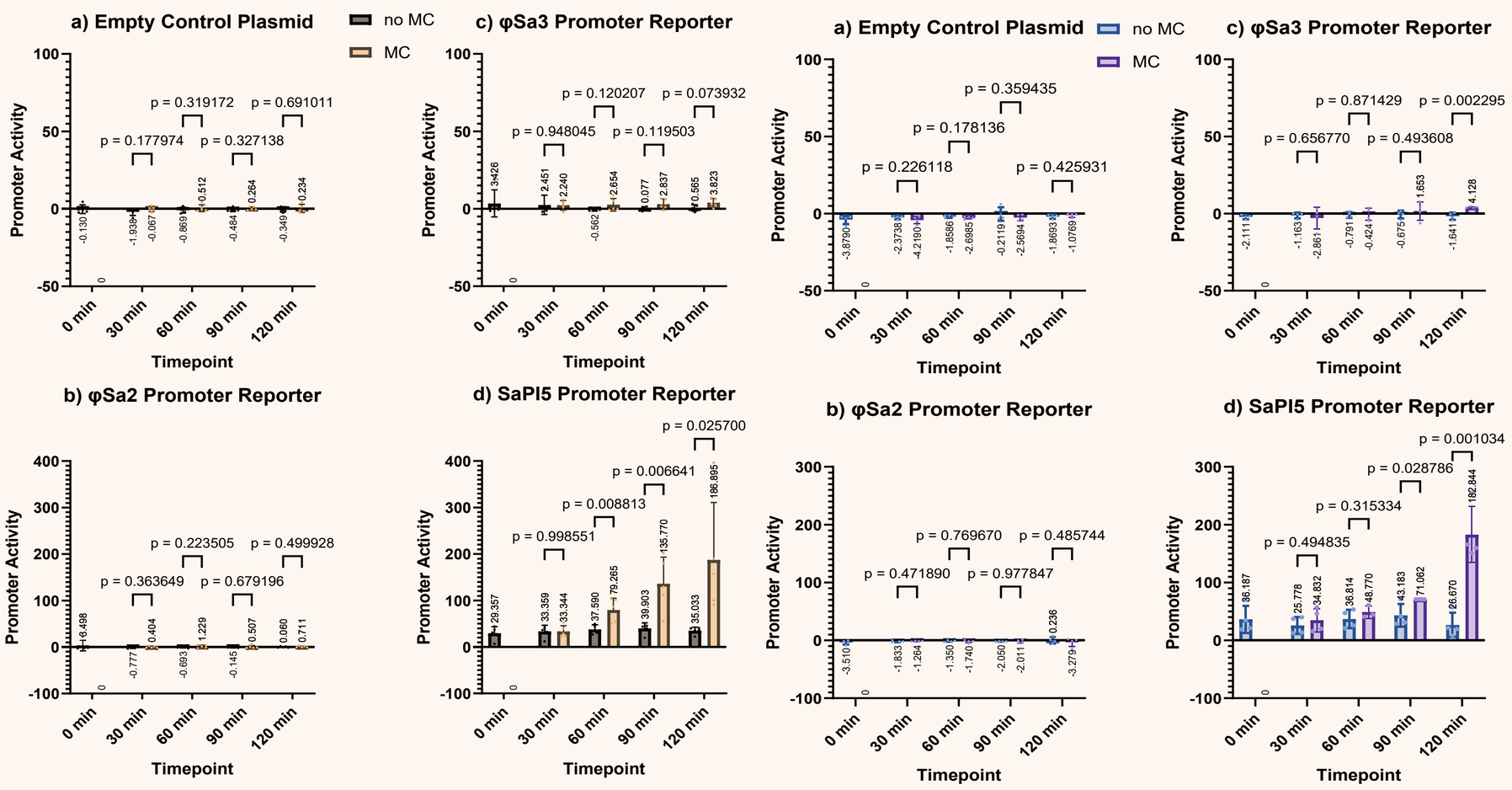


Figure- Promoter Activity of prophages and SaPI5 in RN4220 (left) & USA300 (right).

Legend: Defined strains carrying reporter plasmids were grown to exponential phase, adjusted to OD_{600} ≈ 0.25, & split ± MMC (2 µg/ml). Samples collected 0–120 min. Promoter activity measured by nitrocefin β-lactamase colorimetric assay & normalized to OD_{600}. Bars show mean ± SD of biological repeats (n=5 in RN4220 and n=4 in USA300); dots show individual values. p-values of unpaired t-test (MMC vs. no MC).

CONCLUSION

- SaPI5 shows strongest SOS-induction
- ϕSa3- weak activation
- ϕSa2- inactive, consistent with RNA sequencing results
- Different MGEs follow different induction patterns
- Implications: Strong SaPI5 induction may drive virulence and AMR gene transfer under antibiotic stress

- RN4220 and USA300 show similar trends.
- Control plasmid & ϕSa2 show no significant induction.
- ϕSa3 displays weak induction and significance in USA300 at 120 min.
- SaPI5 exhibited strong MMC induced activation & significance at 90-120 min in both strains.

REFERENCES & Full Paper

ACKNOWLEDGMENT

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