

University of
St Andrews

Investigating Mobile Genetic Element Induction Dynamics in MW2



Orla Rostom

Supervised by Dr Andreas Haag, School of Medicine

BACKGROUND

MW2 is a strain of the bacterium *Staphylococcus aureus* which is resistant to many antibiotics, including methicillin(1). Mobile genetic elements (MGEs) such as prophages and Staphylococcal pathogenicity islands (SaPIs) contribute to the dissemination of antibiotic resistance within the strain(2).

- **Prophages** - viruses integrated into the bacterial chromosome that can carry genes for toxins or immune evasion(3).
- **SaPIs** - phage parasites that hijack phage replication and shape phage-host interactions(4).

When the bacterial SOS response is triggered by DNA damage, prophages and SaPIs can be induced(5), and their lifecycles contribute to the dissemination of antibiotic resistance genes. The nature of their interactions is explored in this study using plasmid-based reporter systems to monitor phage and SaPI promoter activity.

HYPOTHESIS & AIMS

Hypothesis: MGEs are induced differently in clinical and lab MW2 strains.

Aims:

1. Construct plasmid reporter systems
2. Induce and assess responses in RN4220
3. Induce and assess responses in clinical isolate

METHODOLOGY

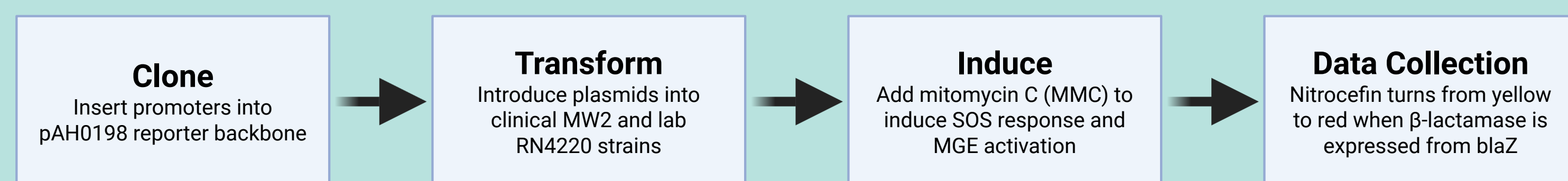


Fig. 1: Flowchart showing methodology

Plasmid design

Reporter constructs formed using pAH0198 cloning backbone which carries a chloramphenicol resistance gene (*cat*) and a β -lactamase reporter (*blaZ*):

- **pAH0874**: ϕ Sa2MW2 *immAR-cro'* promoter region inserted into pAH0198
- **pAH0875**: ϕ Sa3MW2 *cl-cro* promoter region inserted into pAH0198
- **pAH0876**: SaPIMW2 *stl-cro* promoter region inserted into pAH0198

Native MGEs in MW2: ϕ Sa2MW2, ϕ Sa3MW2 and SaPIMW2.

Nitrocefin Assay

Bacterial cultures were grown in tryptic soy broth with appropriate antibiotics at 37°C until mid-exponential phase. Samples were snap-frozen after dilution in phosphate buffer. β -lactamase activity was quantified using a nitrocefin assay, where hydrolysis of substrate causes a colour change. Absorbance was measured over 30 minutes using a microplate reader and used to calculate promoter activity.

ACKNOWLEDGMENTS

I would like to thank my supervisor, Dr Andreas Haag, for his guidance and patience throughout this research project. I would also like to thank my lab partner, Ishani Badala, for her collaboration and insight into the project. Thank you to Lord Laidlaw and the Laidlaw Foundation for funding this research and supporting me throughout.

REFERENCES

Scan the QR code to view references, the research project this poster was based on, and other related materials:



Data was filtered by removing extreme values where bubbles were present in the plate reader. Promoter activity was calculated using OD, slope per hour, dilution factor and volume. Mean promoter activity was taken across the 4 repeats at each timepoint. Welch's two-tailed t-test was used to calculate whether there had been any significant increase in promoter activity at each timepoint. Significant results are marked with an asterisk. Error bars represent standard deviation.

NON-CLINICAL RESULTS

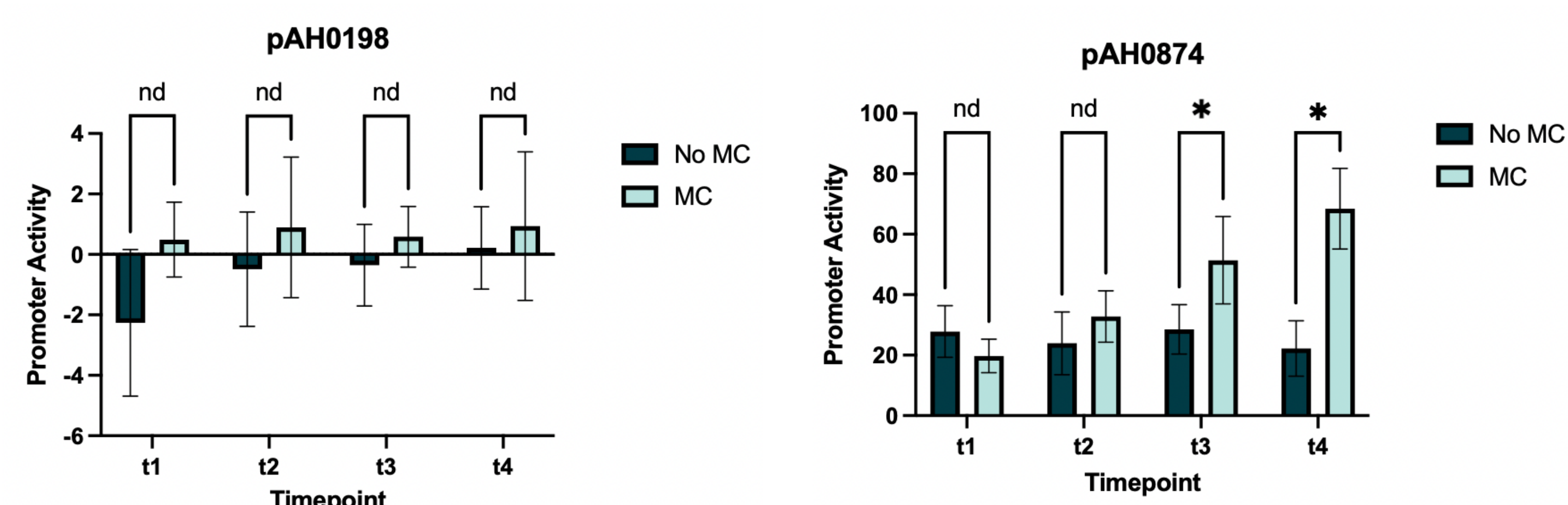


Fig. 2: Graph showing results of Welch's two-tailed t-test for RN4220 pAH0198.

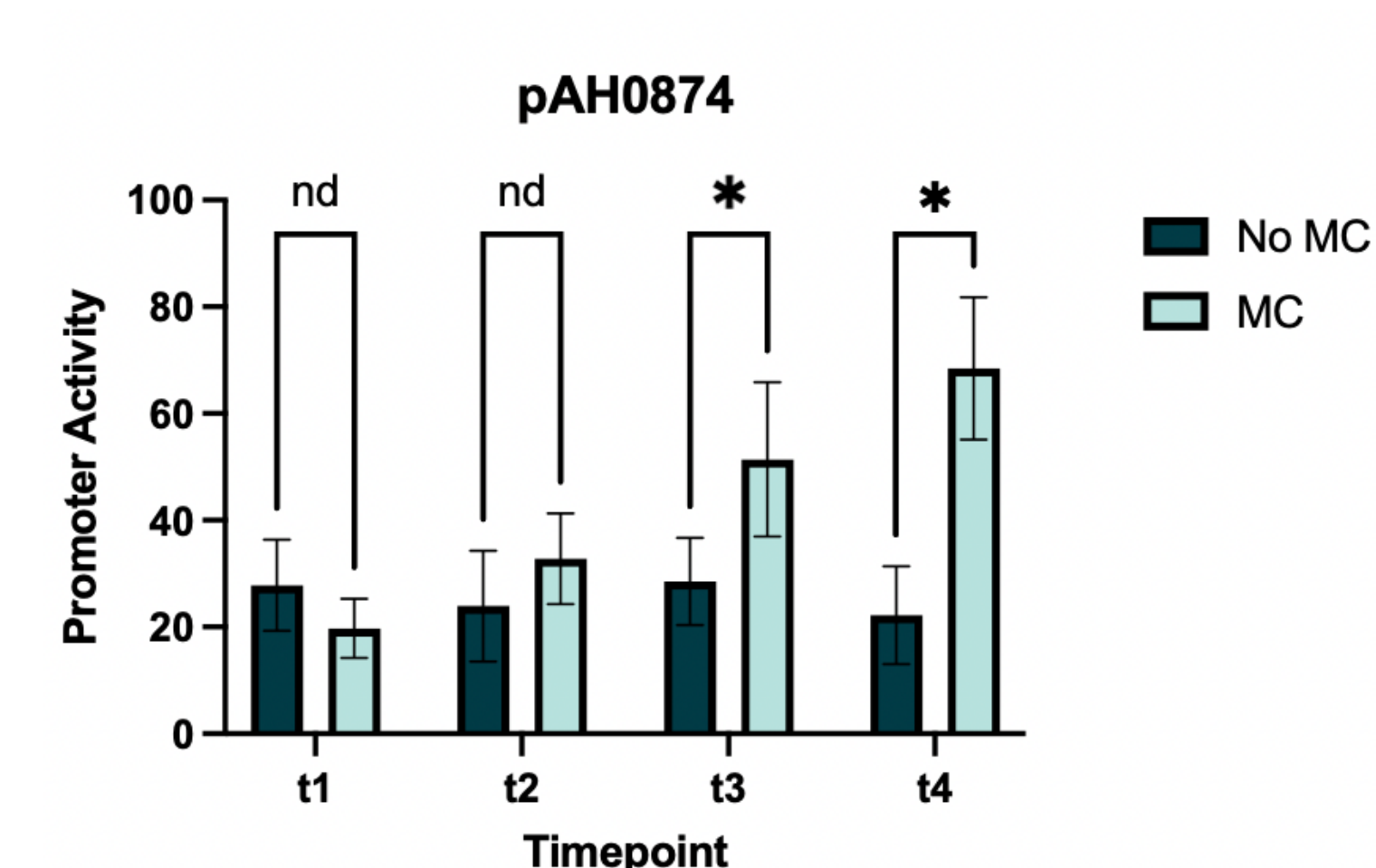


Fig. 3: Graph showing results of Welch's two-tailed t-test for RN4220 pAH0874.

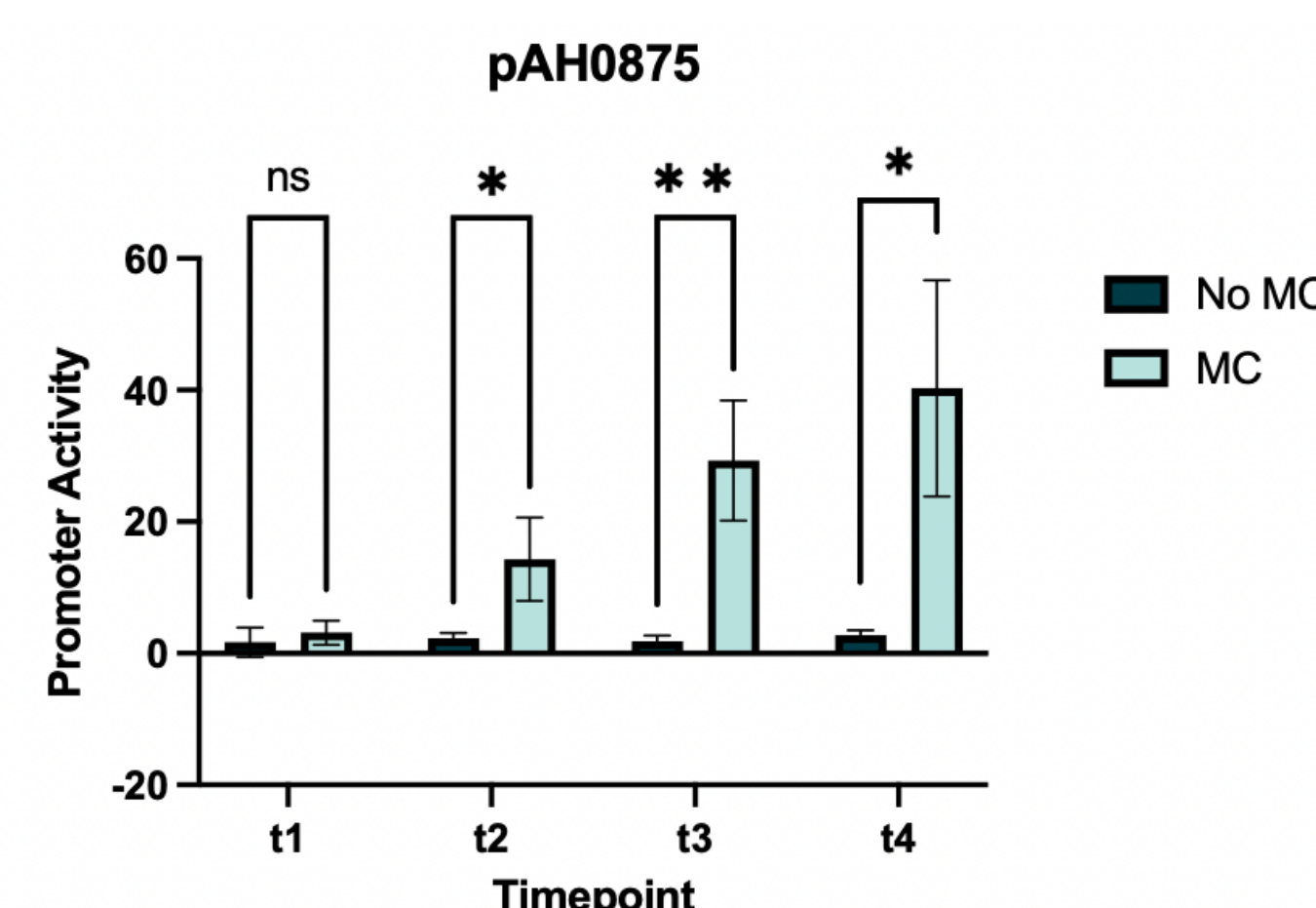


Fig. 4: Graph showing results of Welch's two-tailed t-test for RN4220 pAH0875.

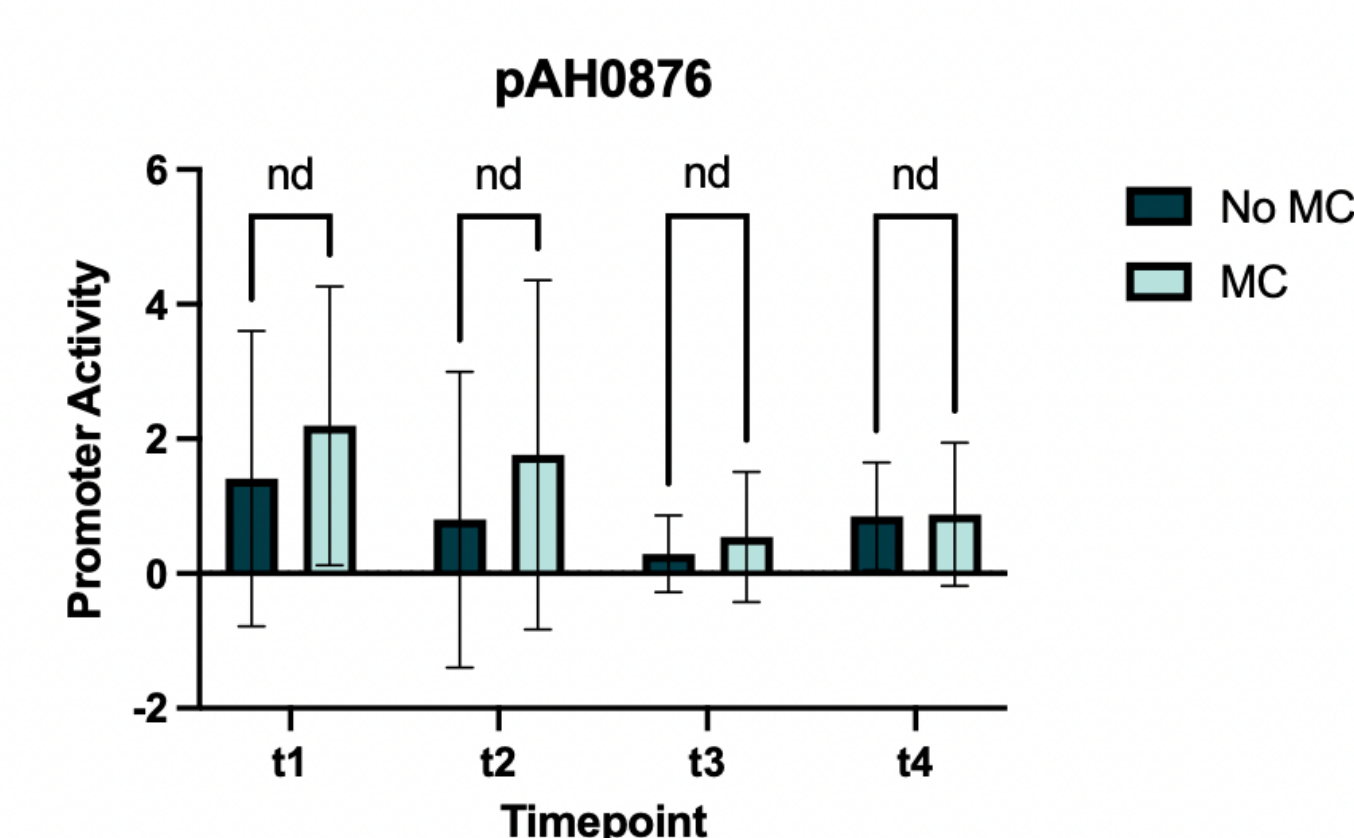


Fig. 5: Graph showing results of Welch's two-tailed t-test for RN4220 pAH0876.

CLINICAL RESULTS

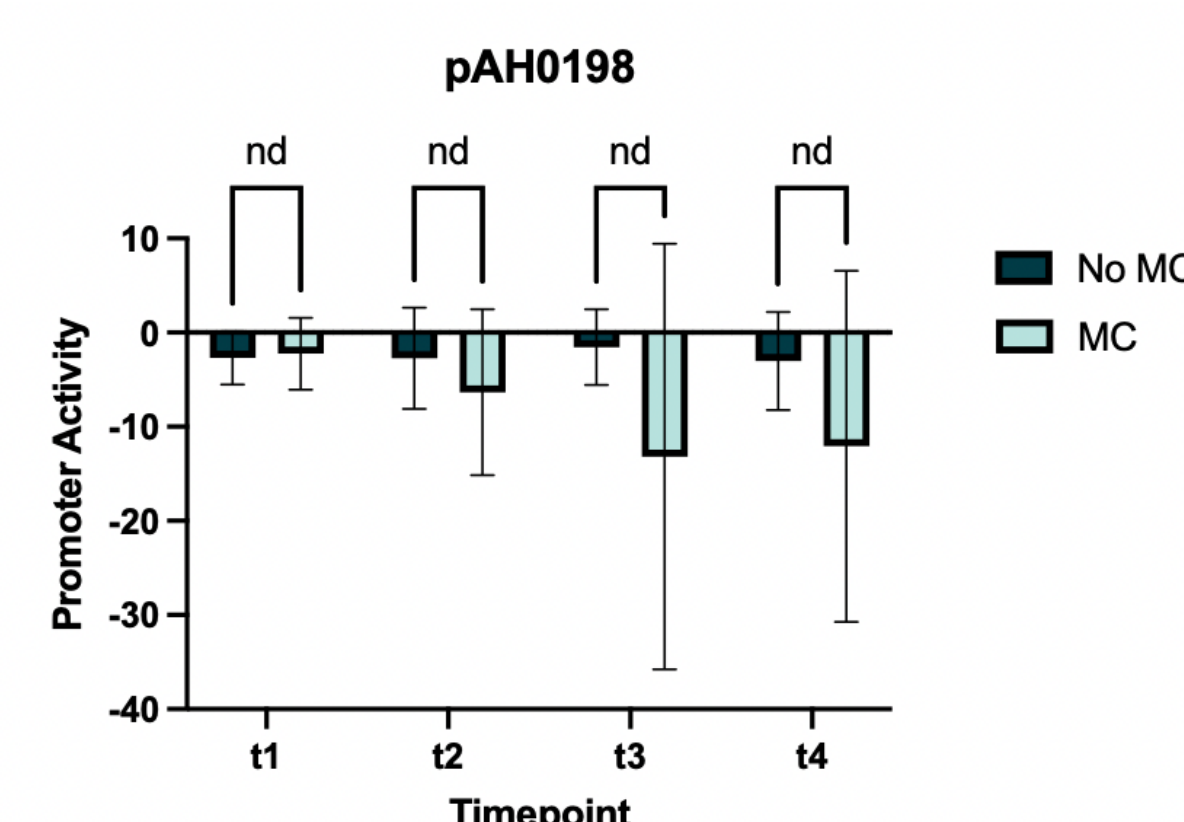


Fig. 6: Graph showing results of Welch's two-tailed t-test for clinical pAH0198 strain.

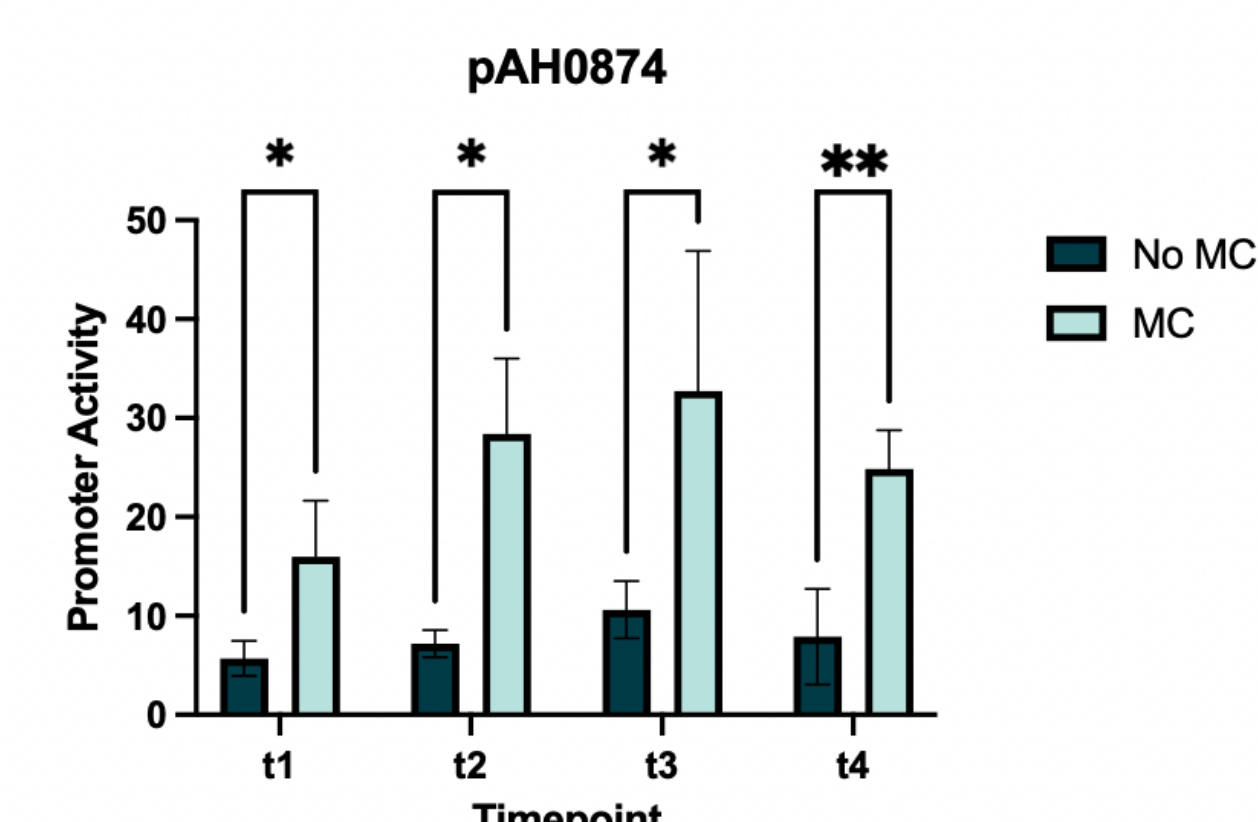


Fig. 7: Graph showing results of Welch's two-tailed t-test for clinical pAH0874 strain.

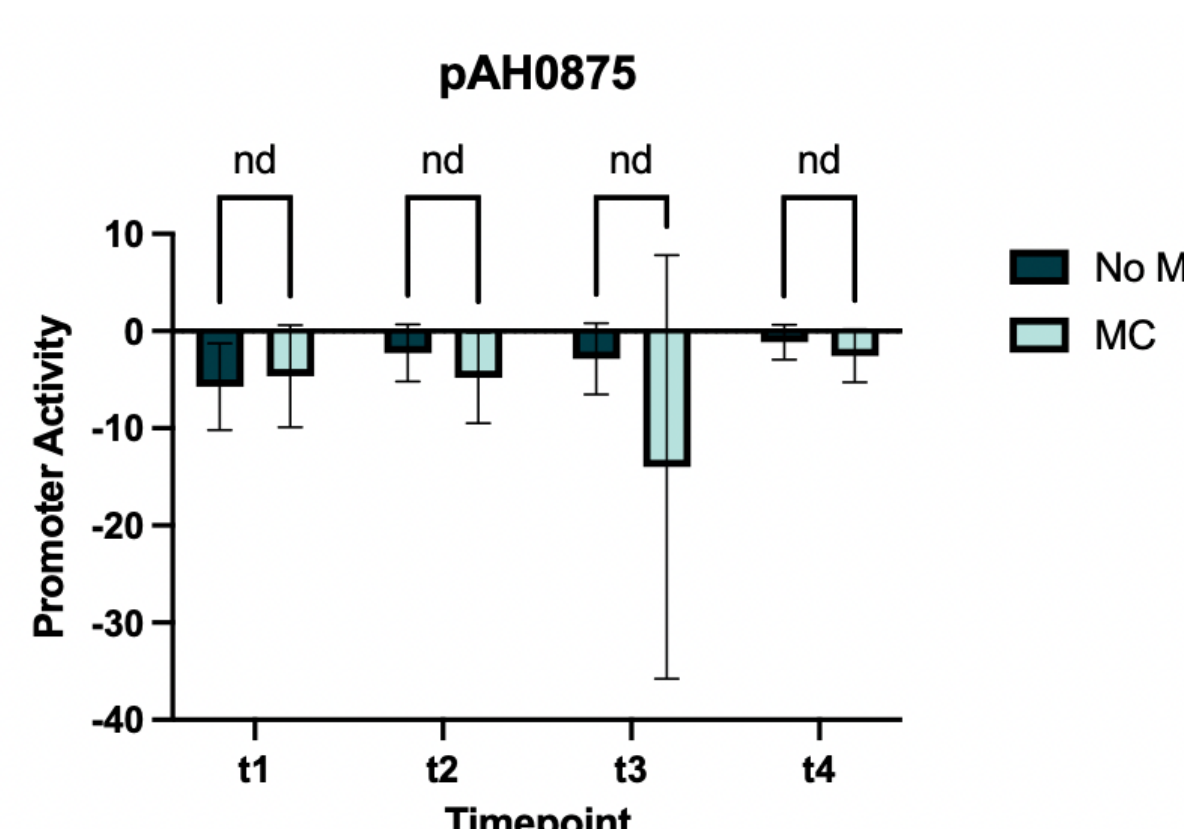


Fig. 8: Graph showing results of Welch's two-tailed t-test for clinical pAH0875 strain.

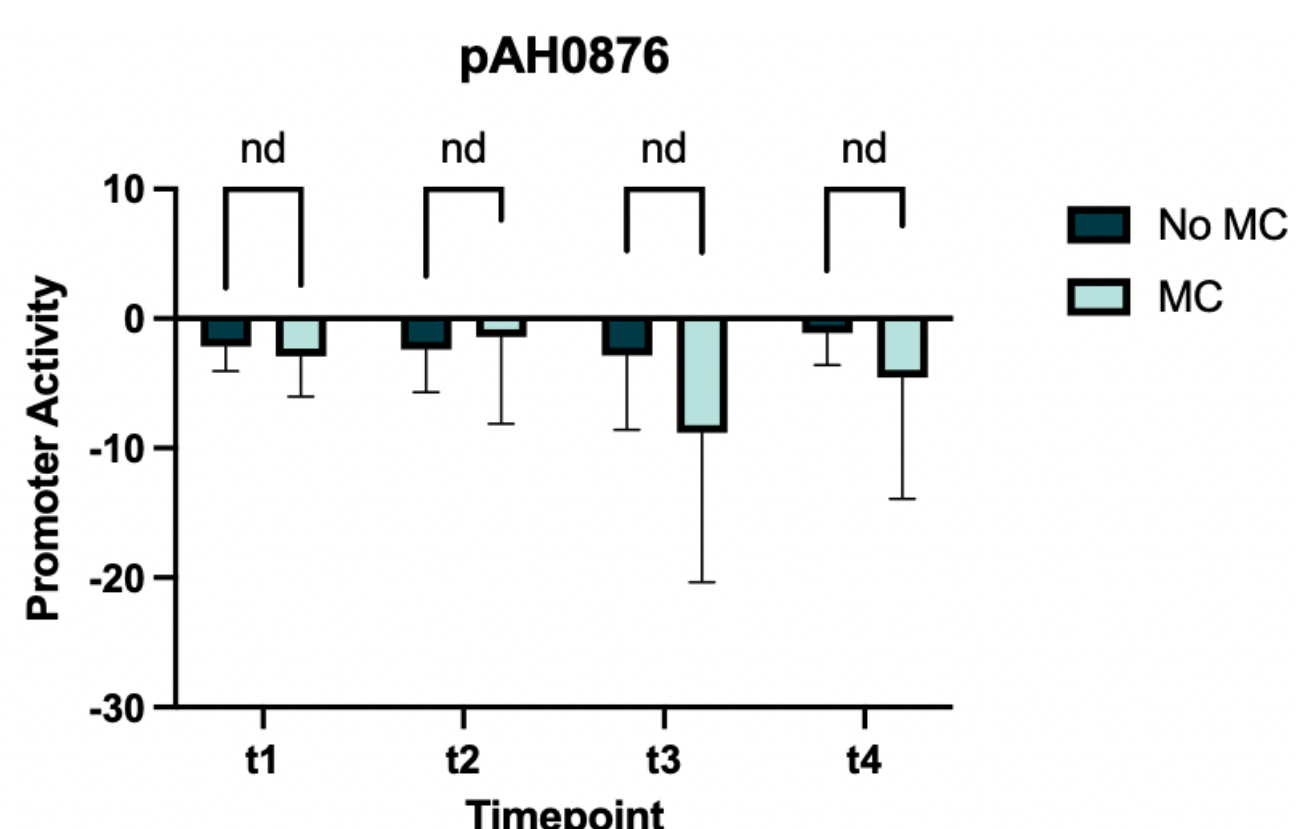


Fig. 9: Graph showing results of Welch's two-tailed t-test for clinical pAH0876 strain.

CONCLUSIONS

- ϕ Sa2MW2 (pAH0874): induced in both strains; increasing over time.
 - ϕ Sa3MW2 (pAH0875): induced in lab strain only.
 - Control (pAH0198) & SaPIMW2 (pAH0876): no induction.
- Induction is element- and strain-specific, suggesting context-dependent regulation. Future work will test regulators and environmental triggers.