

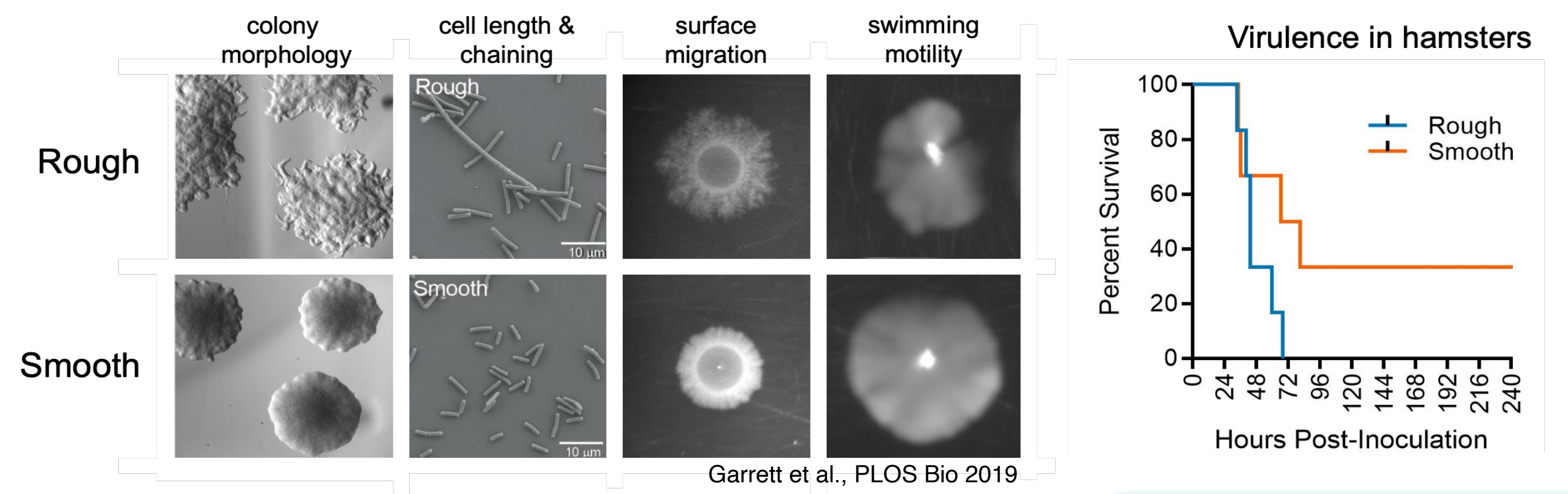
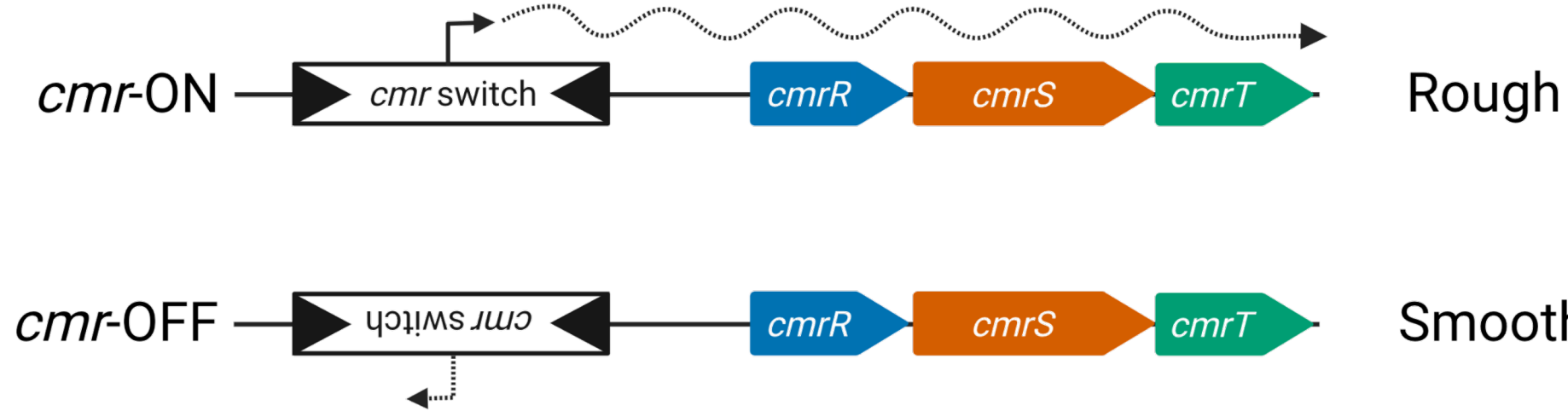
Identifying genes that affect colony morphology in bacterial pathogen *Clostridioides difficile*

Aicha Kaouss, Anchal Mehra, Rita Tamayo, PhD

Department of Microbiology & Immunology, University of North Carolina at Chapel Hill

Phenotypic heterogeneity and virulence are linked in *C. difficile*

- C. difficile* is a leading cause of healthcare-associated infections, and community-acquired cases are rising.
- Symptoms range from mild diarrhea to fatal pseudomembranous colitis and toxic megacolon.
- C. difficile* exhibits phenotypic heterogeneity through phase variation - a mechanism in which a bacterium can reversibly switch expression of specific genes on and off.
- Phase variation serves as a bet-hedging strategy to improve odds of survival in the presence of a changing or stressful environment.
- The *cmr* switch, one of eight known phase variable elements in *C. difficile*, modulates expression of *cmrRST*, which encodes an atypical signal transduction system.



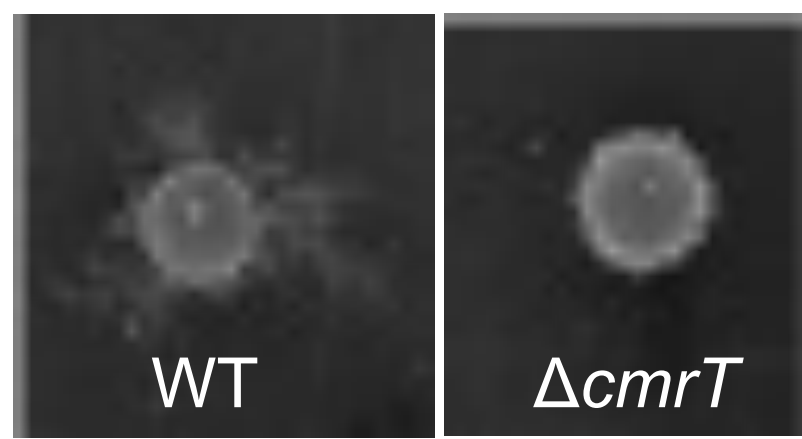
- Expression of *cmrRST* results in the formation of rough colonies, cell chaining, decreased swimming motility, increased surface motility, biofilm formation, and increased virulence in the hamster model of infection.
- Our group is taking multiple approaches to identify the genes that affect these phenotypes: RNA-Seq, suppressor screens, and transposon mutagenesis

Research Goal:
Identify genes important for rough or smooth colony formation via:

- Suppressor Screen
- Transposon Mutagenesis

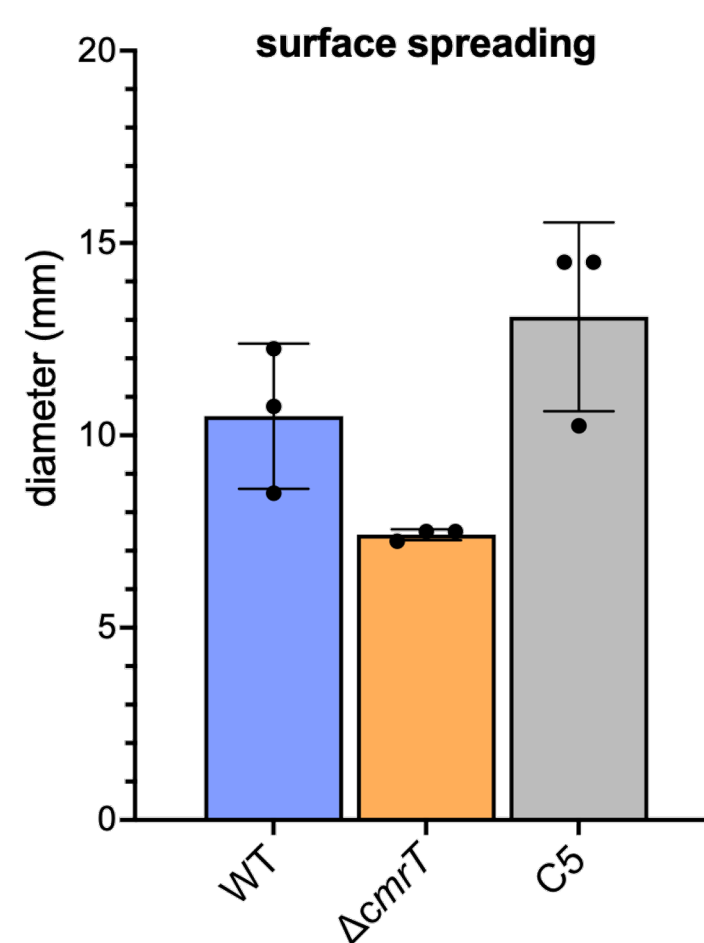
A suppressor mutation restores rough colony morphology in the $\Delta cmrT$ strain

- Using the $\Delta cmrT$ strain, which cannot form rough colonies or spread across an agar surface, we screened for suppressors that restored rough colony morphology and surface spreading after extended incubation on agar.



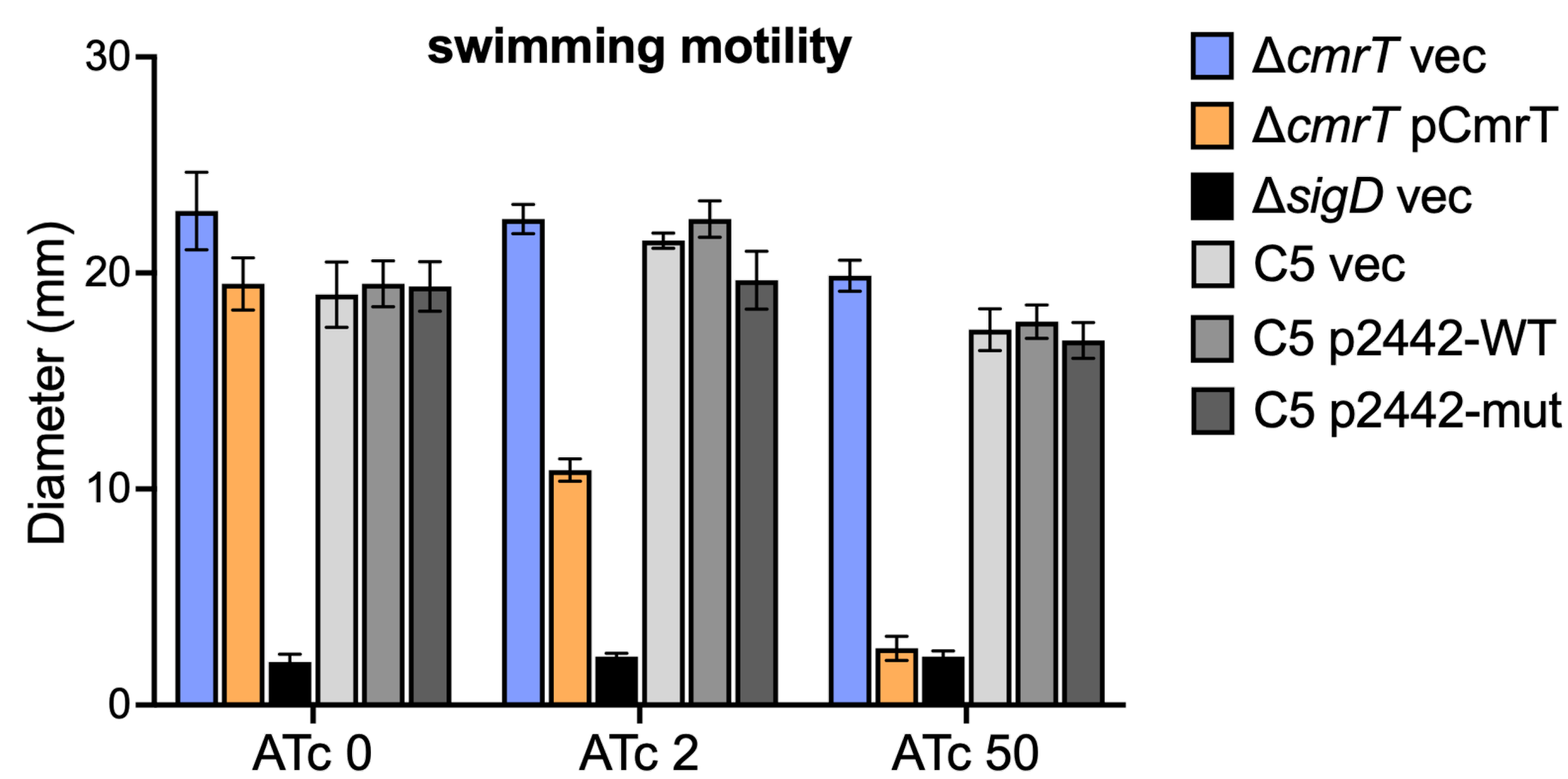
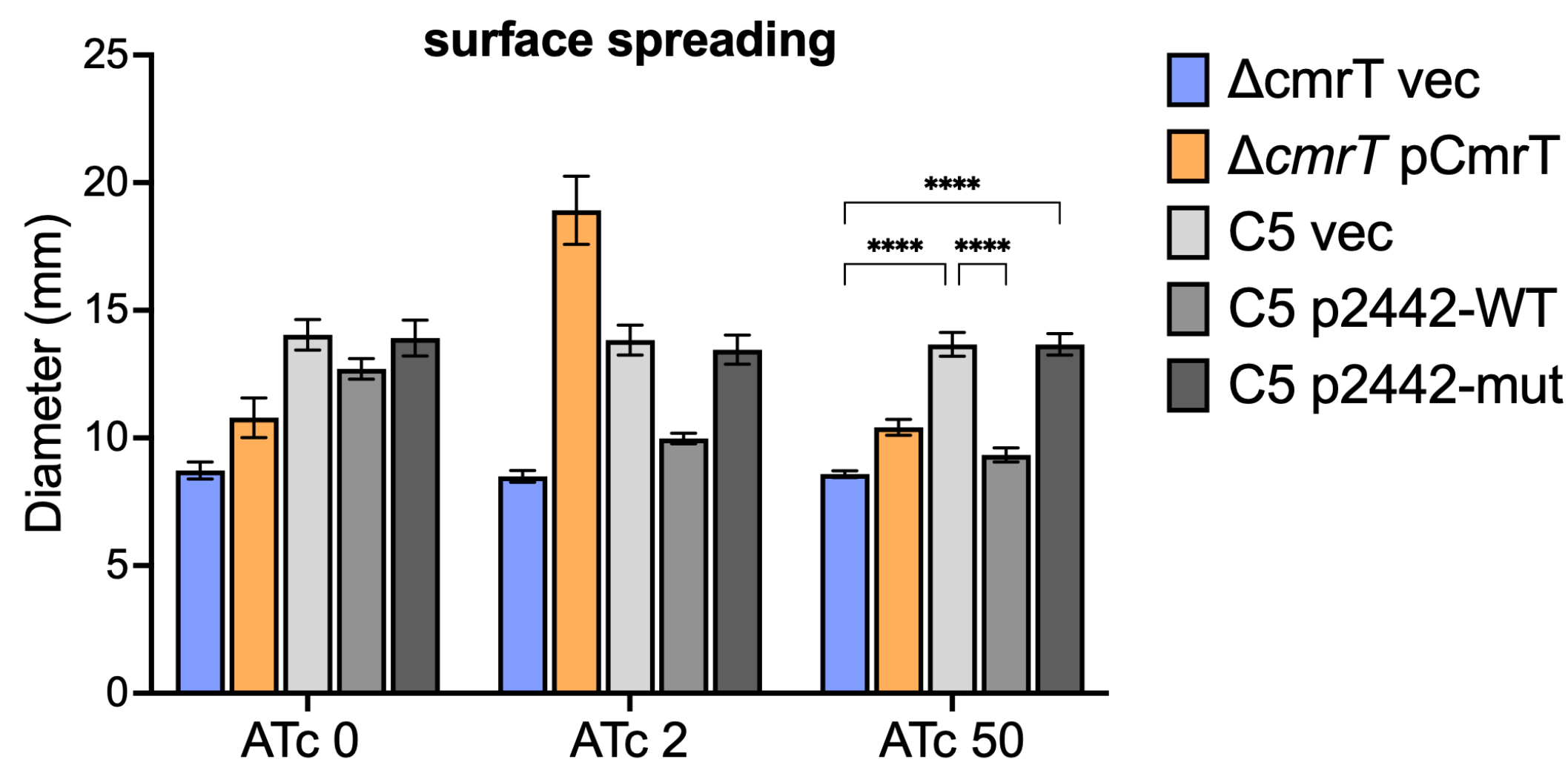
Garrett et al., PLOS Bio 2019

- We isolated suppressor "C5", which:
 - Forms rough colonies
 - Spreads across a surface
 - Forms chains of cells
- Whole genome sequencing of C5 revealed a nonsense mutation in the gene CDR20291_2442.

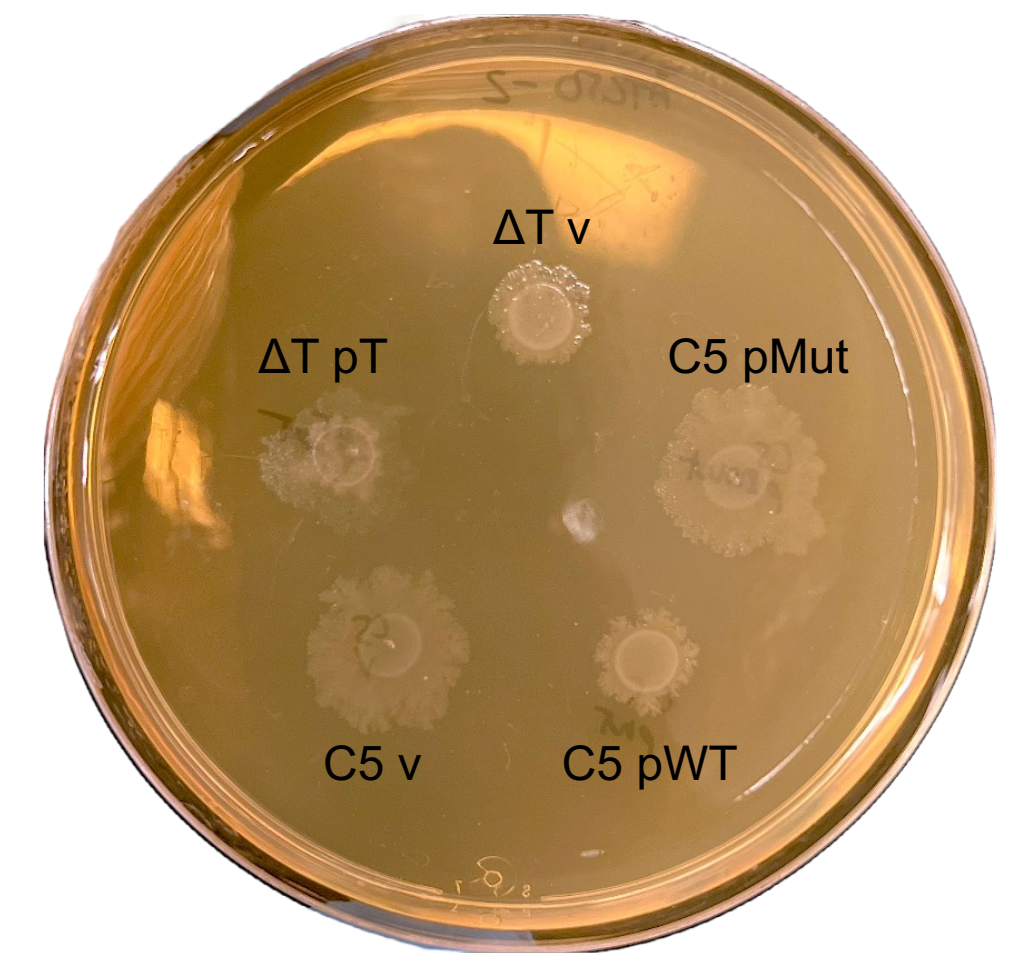


WT
ATGAAGATAGATTTTGAATGATTGAACTA'
M K I D L E M I E L

C5
ATGAAGATAGATTTTGAATGATTGAACTA'
M K I D L E M I * L



Wild-type CDR20291_2442 may function to maintain smooth colony morphology



- Complementing a functional, wild-type version of CDR20291_2442 into C5 restores $\Delta cmrT$ phenotype.
- cmr*-ON-associated surface spreading correlates with decreased swimming motility. However, a swimming motility assay in the C5 suppressor revealed no significant difference in swimming motility between C5 suppressor and $\Delta cmrT$.
- These findings suggest that swimming motility is regulated by a separate mechanism than surface motility in the C5 suppressor.

Using transposon mutagenesis to find isolates that restore rough colony morphology

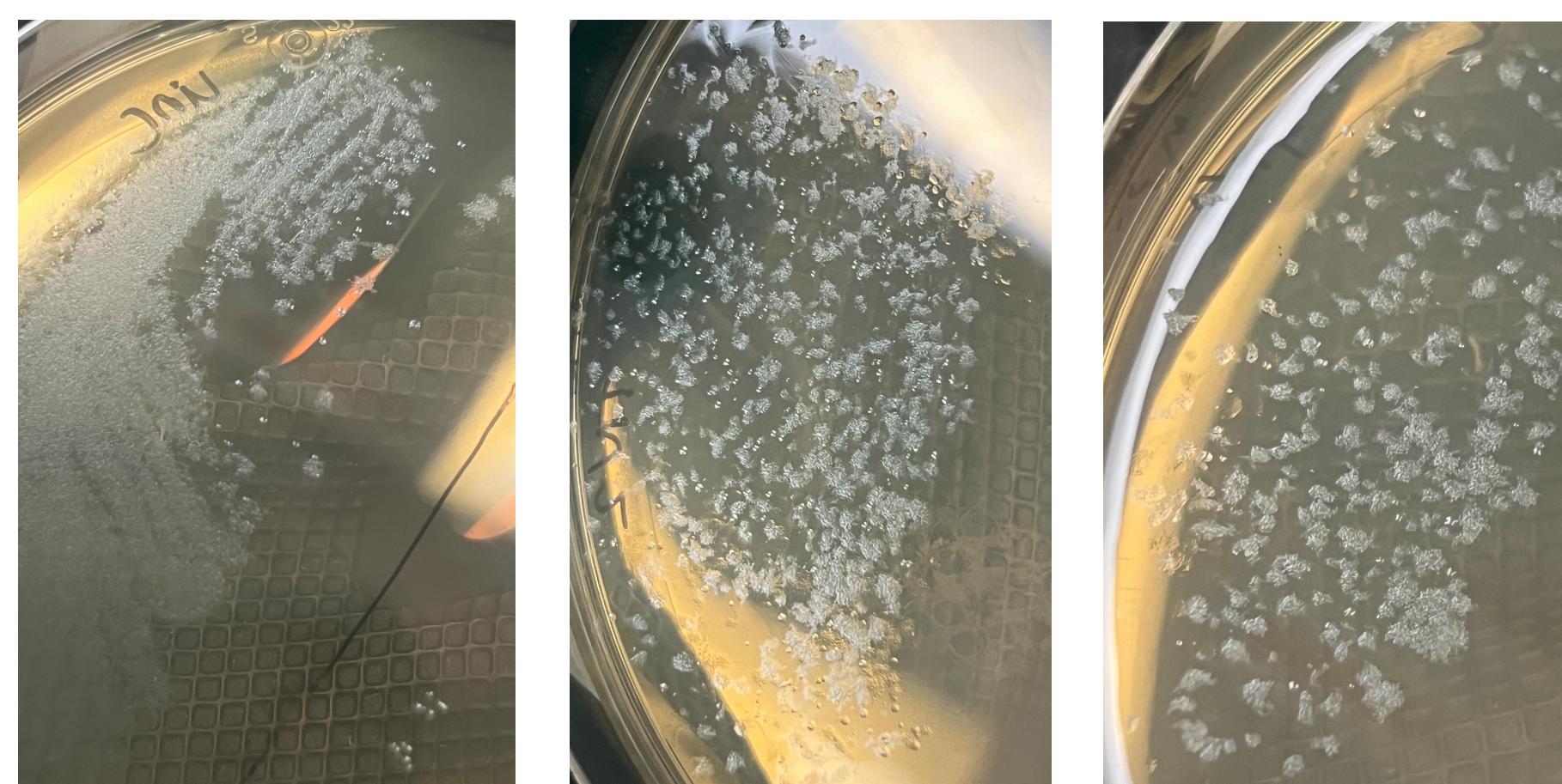
Approach:

- Plasmid carrying transposase and transposon inserted in $\Delta cmrR\Delta cmrT$ strain (forms smooth colonies)
- Transposition induced
- Screen for rough colonies
- Rough colony isolates plated on antibiotics to ensure transposon insertion into genome

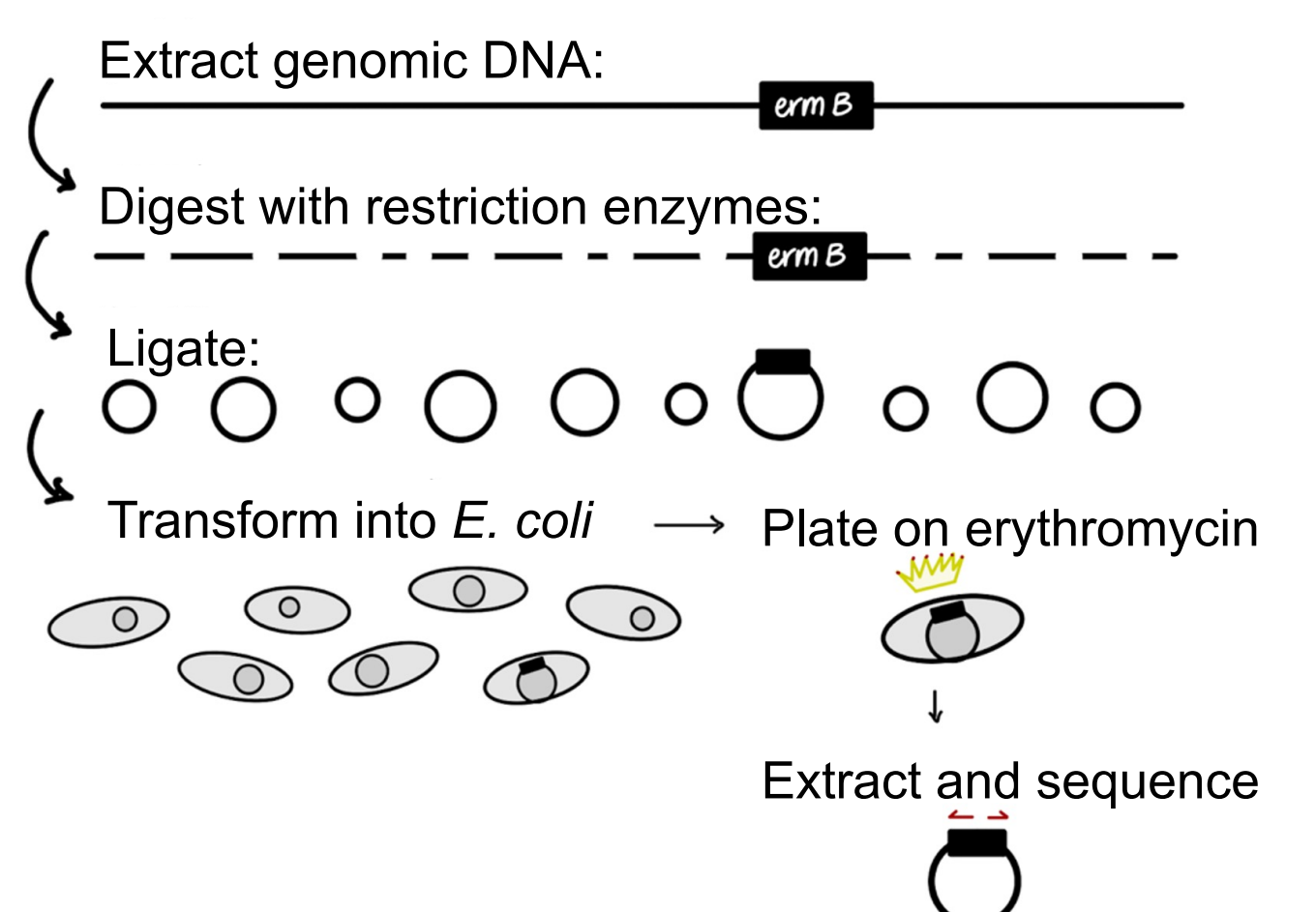


Garrett et al., mBio 2022

We identified ~30 colony isolates that displayed rough colony morphology.



Future work will identify the sites of transposon insertion



References

- Garrett EM, Sekulovic O, Wetzel D, Jones JB, Edwards AN, Vargas-Cuevas G, et al. (2019) Phase variation of a signal transduction system controls *Clostridioides difficile* colony morphology, motility, and virulence. *PLoS Biol* 17(10): e3000379.
- Garrett EM, Mehra A, Sekulovic O, Tamayo R. Multiple Regulatory Mechanisms Control the Production of *CmrRST*, an Atypical Signal Transduction System in *Clostridioides difficile*. *mBio*. 2021 Feb 22;13(1):e0296921.

Acknowledgements

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