# Comparison of the genetic basis of biofilm formation between *Escherichia coli* and *Salmonella enterica* serovar Typhimurium

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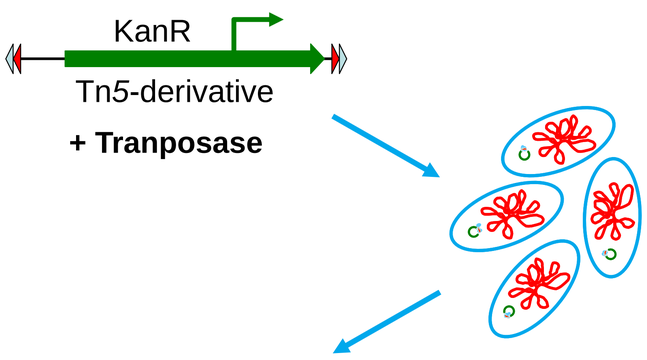
## Bacteria often exist in structured aggregated communities called biofilms

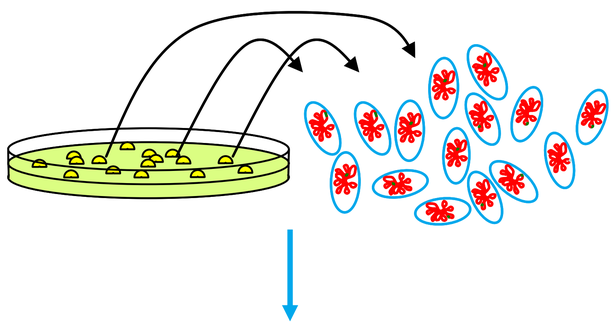
- Biofilms complete a lifecycle where cells aggregate, grow and produce a structured community before dispersing
- Progression through this lifecycle requires controlled temporal gene expression to maximise fitness at each stage.
- We recently described the genes required for biofilm formation in *E. coli* BW25113 over time and showed how temporal control of gene expression is necessary for optimal fitness across the biofilm life cycle *(Holden et al 2021, Microbial Genomics)*
- This work used TraDIS-*Xpress*; a massively parallel transposon mutagenesis approach using transposon-located promoters to assay both essentiality and impacts of altered expression of all genes in a genome.
- We have repeated this work with *Salmonella enterica* serovar Typhimurium 14028S allowing a comparison of important pathways over time between the species.

## Experimental setup

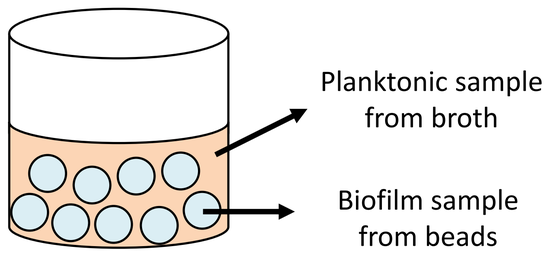
**Introduce transposon into cells (with outwards-facing inducible promoter)**



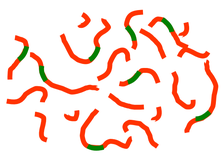

**Select successful transposon mutants (approx. 500,000-1million per library)**



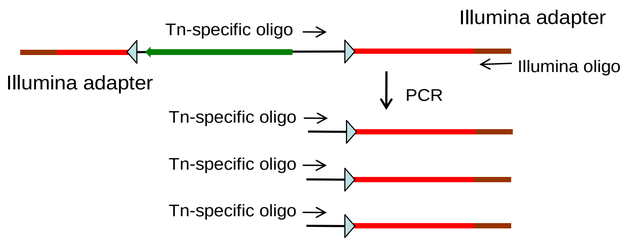
**Grow mutant library in broth with glass beads**

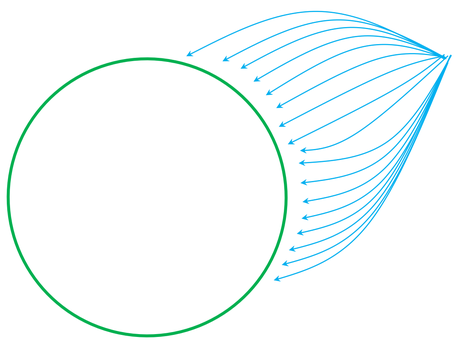



**Sample both biofilm and planktonic conditions after 12, 24 and 48 hours growth**



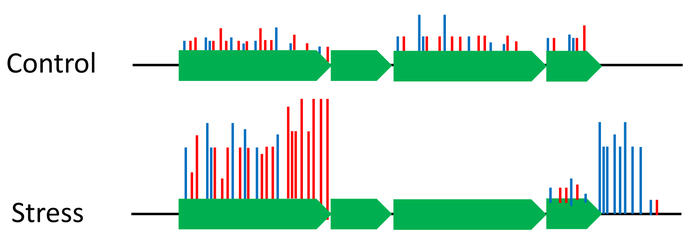
**Extract DNA + Sequence with transposon-specific primers**



**Map sequencing reads to bacterial reference genome**

**Compare insertion profiles of planktonic and biofilm conditions at each time point**




## Supported by

MICROBIOLOGY SOCIETY

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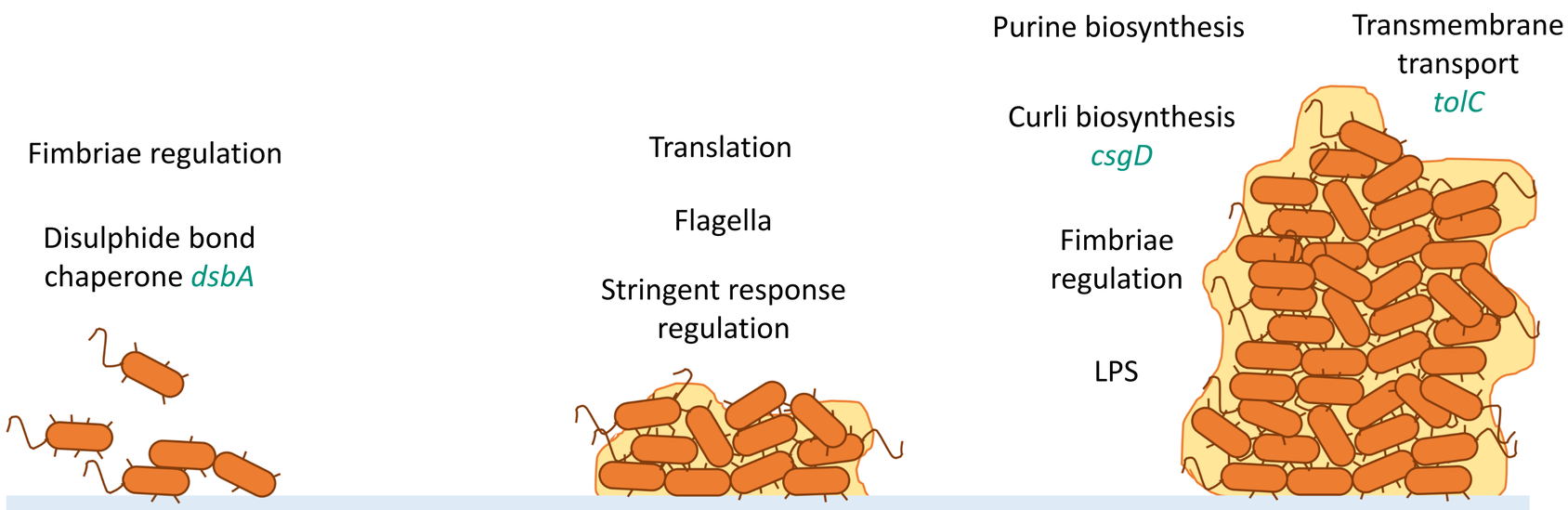
## Functions of genes involved in *E. coli* and *S.* Typhimurium biofilm formation through time

**48 genes in *E. coli* and 78 genes in *S.* Typhimurium were identified to affect the fitness of the biofilm over time**




**More important in *E. coli* biofilms**

Cellulose biosynthesis regulation

Motility regulation

Amino acid biosynthesis

**More important in *S.* Typhimurium biofilms**

DNA housekeeping

cAMP biosynthesis

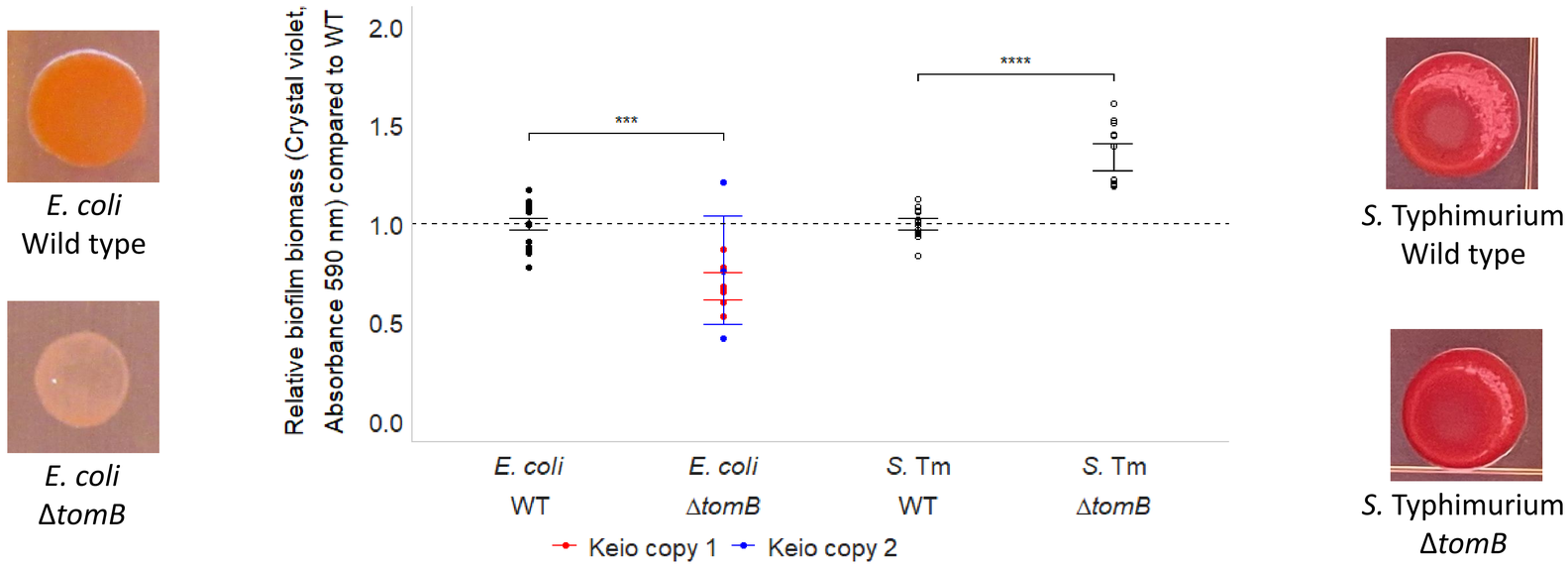
Cell division

c-di-GMP regulation

Respiration

## Differences between *E. coli* and *S.* Typhimurium biofilm development

- Different roles were predicted for TomB in *E. coli* (inactivation bad for biofilm development) and *S.* Typhimurium (inactivation good for biofilm development)
- TomB is the antitoxin to Hha - Hha has been shown to reduce biofilm development by repressing fimbriae production, inducing cell lysis and biofilm dispersal *(Garcia-Contreras et al 2008 PLoS One)*




***E. coli ΔtomB***
↓ curli
↓ biofilm biomass

***S.* Tm Δ*tomB***
↑ biofilm biomass

## Summary

- Core pathways for biofilm formation in both *E. coli* and *S.* Typhimurium include curli biosynthesis, purine biosynthesis, transmembrane transport, LPS biosynthesis, chaperones and fimbriae regulation
- Genes involved in respiration were identified to affect the fitness of *S.* Typhimurium biofilms, but phenotypic analysis found these genes benefitted biofilm formation in *E. coli* as well
- Novel roles for five genes in biofilm formation were found in *E. coli* and 21 genes in *S.* Typhimurium
- TomB was found to act in opposite ways between the species, the role of interaction with H-NS is being investigated
- Future work = characterising the novel genes, comparing different strains and different species, and testing different conditions