Reverse genetic analysis of the bacterial helicase loader protein DnaI

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ABSTRACT

DNA replication initiation is essential for genome duplication. To initiate DNA replication, a helicase must be loaded at an origin to unwind DNA so that DNA synthesis may begin. Despite the universal importance of helicase loading, crucial aspects of the process in bacteria are poorly understood. In particular the physiological relevance of a recently proposed interaction between the helicase loader protein and single-strand DNA, based on cryo-EM structures *in vitro*, is unclear. The goal of this project is to address this question *in vivo,* using the well understood model system of *Bacillus subtilis* to determine whether the findings *in vitro*[1] reflect those in the biological system. The approach employs new tools developed in the Murray lab that allow the construction and characterization of mutations within the essential helicase loader protein, to disrupt its original sequence and observe effects on the viability of *B. subtilis.*

AIMS

- Identify **homologous** residues in *B. subtilis* helicase loader DnaI that correspond to those previously described in the *E. coli* helicase loader DnaC that makes direct **contacts to single stranded DNA [1]**.
- Introduce **mutations** that change each these residues in DnaI (T237, W239, L208) to **alanine** using vector plasmid pCW200.
- **Transform** a strain of *B. subtilis* which is engineered to express a wild-type *dnaI* gene in the presence of IPTG and the mutated *dnaI* gene in the absence of IPTG.
- Observe **viability** of mutants in +/- IPTG media to determine the effect of alanine substitution at these residues.

METHODS

Mutant plasmid preparation and purification

- Mutations were made via **QuickChange mutagenesis** onto a vector plasmid containing resistance to the antibiotic **kanamycin to allow selection**. This plasmid was **transformed** into *E. coli* to increase yield as shown in Figure 1.

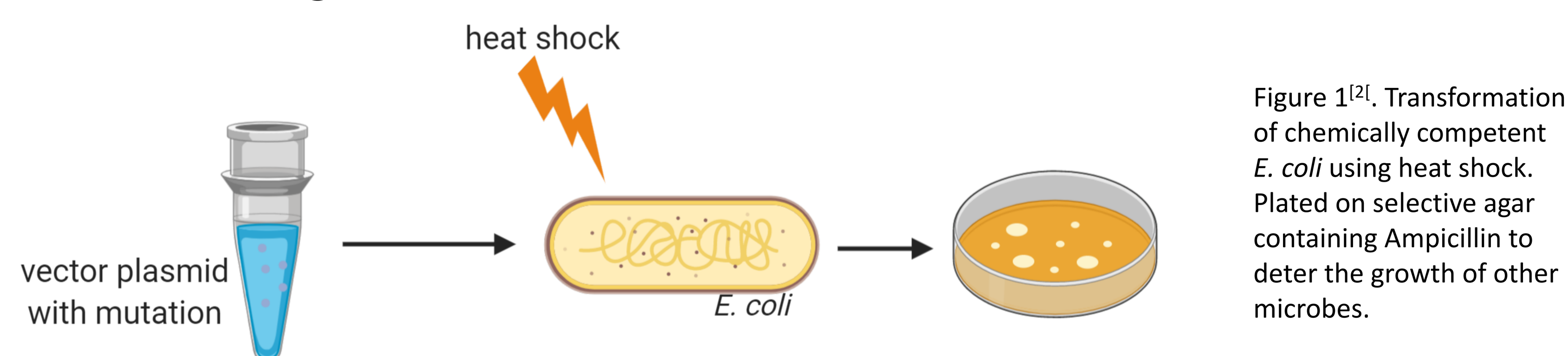

Figure 1[2]. Transformation of chemically competent *E. coli* using heat shock. Plated on selective agar containing Ampicillin to deter the growth of other microbes.

- **Purification** of plasmid using QIAGEN miniprep kit.
- The plasmid was **sequenced** to ensure integrity of the mutation and plasmid sequence.

Transformation of *B. subtilis*

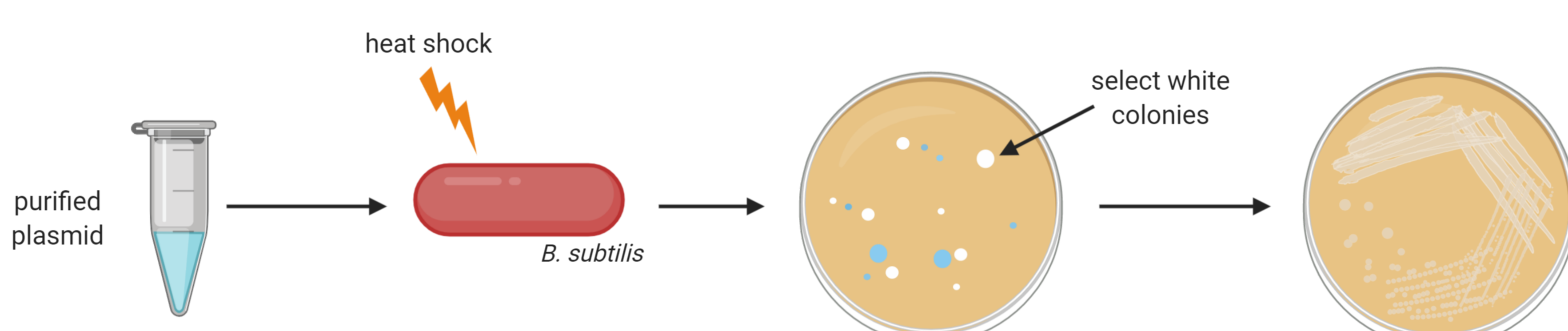

Figure 2[2]. Transformation of chemically competent sCW234 *B. subtilis* using heat shock. Selection of living transformant colonies and re-streaking.

- **Transformation** of *B. subtilis* with miniprepped plasmid.
- **Blue-white screening** was used to identify colonies containing the mutation as per **homologous recombination** in Figure 3.

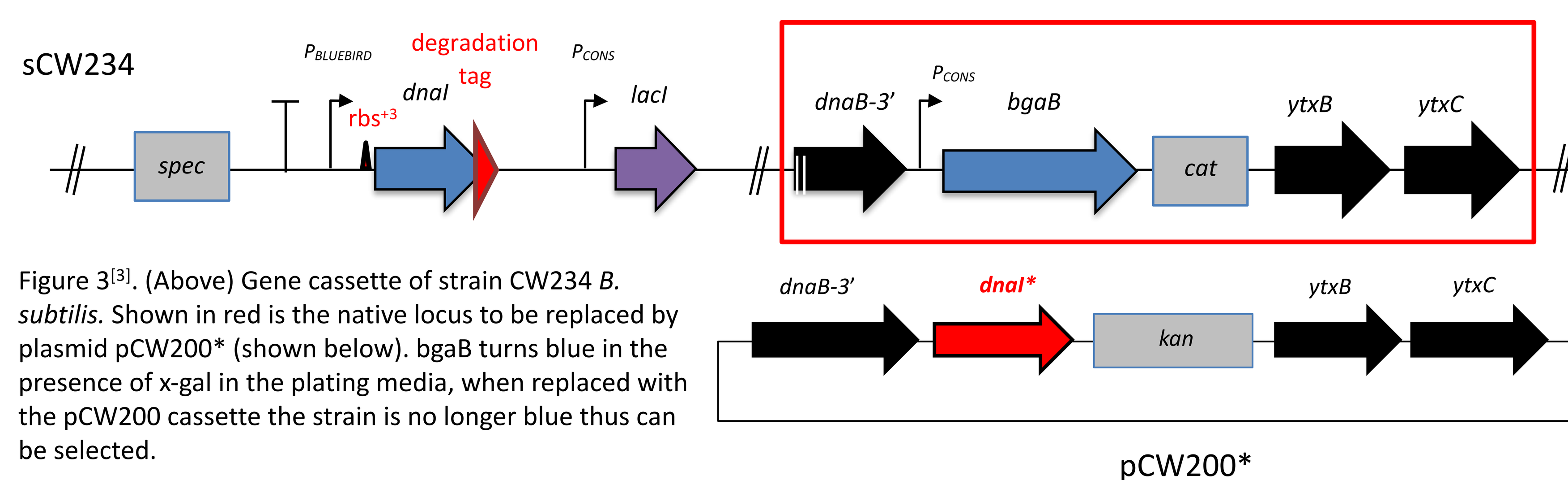

Figure 3[3]. (Above) Gene cassette of strain CW234 *B. subtilis.* Shown in red is the native locus to be replaced by plasmid pCW200* (shown below). bgaB turns blue in the presence of x-gal in the plating media, when replaced with the pCW200 cassette the strain is no longer blue thus can be selected.

Spot Titre Assay

- 5µL of each mutant *B. subtilis* colony was plated at **10-fold dilutions** as a spot against positive control strain sCW221 (parent of all strains used) and negative control strain sCW234 to show **strain viability**.

RESULTS

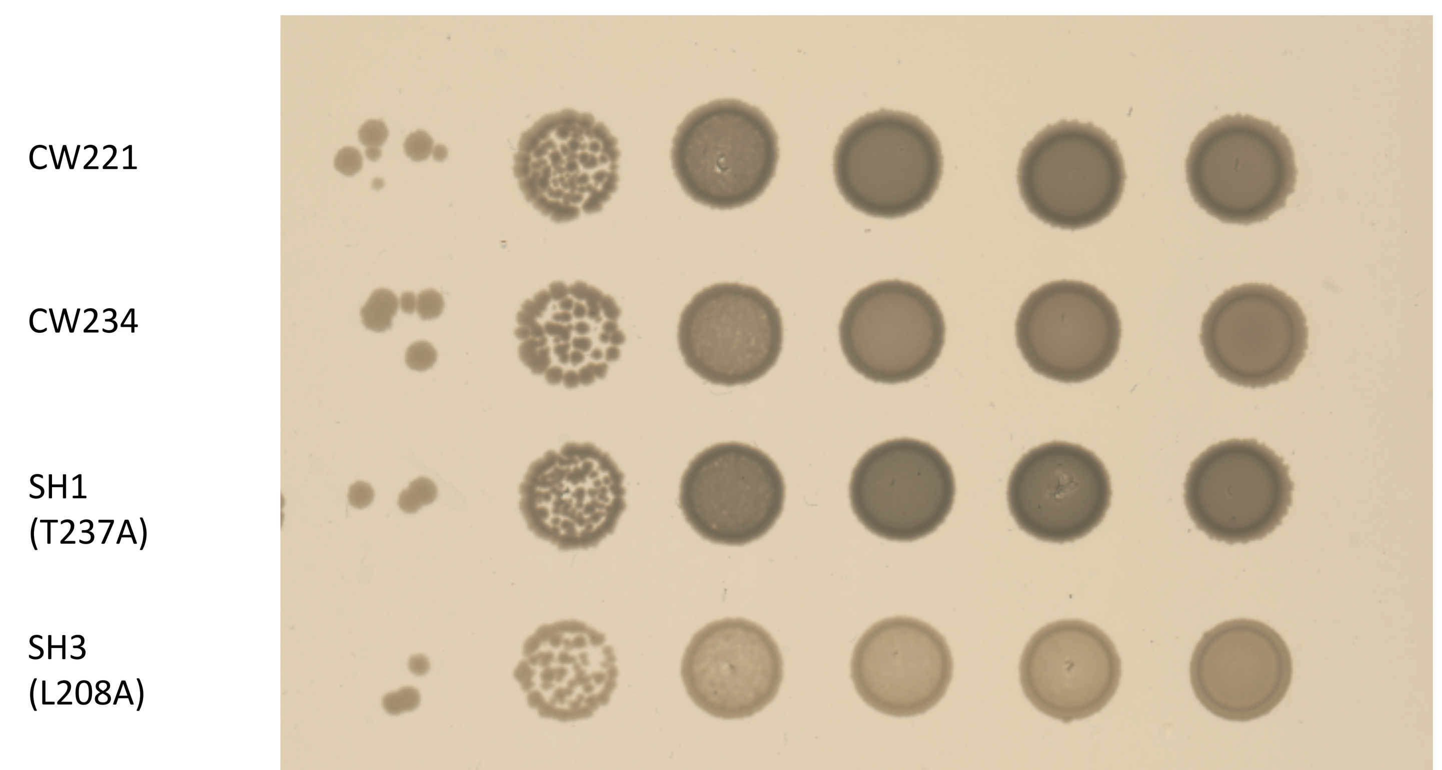

Figure 4. A spot titre assay of *B. subtilis* strains, plated on nutrient agar containing **IPTG** and expressing wild-type DnaI along with DnaI mutants.

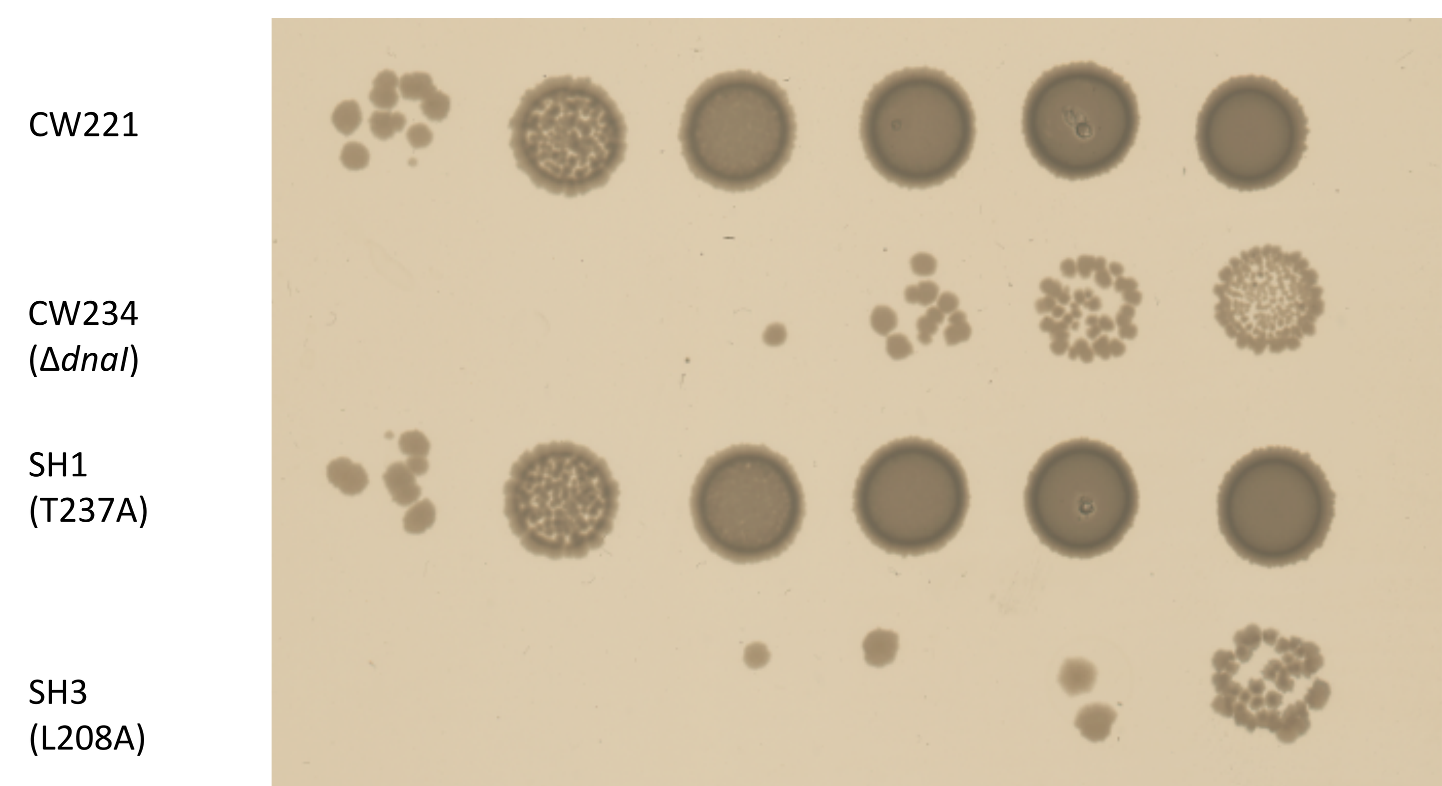

Figure 5. A spot titre assay of *B. subtilis* strains, plated on nutrient agar –IPTG. Showing normal growth of SH1 (T237A mutation) as observed with positive control CW221 and significantly decreased growth of SH3 (L208A mutation) as observed with negative control strain CW234 (Δ*dnaI*).

DISCUSSION

- When plated on nutrient agar containing IPTG all strains grow due to induction of the ectopic locus where there is the wild-type *dnaI* gene (Figure 3, 4).
- When plated on nutrient agar without IPTG (Figure 5) the effect of the mutation in the native *dnaI* can be observed.
- SH1 containing mutation of threonine to alanine at residue 237 **grows normally** in the presence and absence of IPTG. This suggests that **T237 is not an essential residue in DnaI** for contacting single stranded DNA and initiating DNA replication in model system *B. subtilis.*
- SH3 containing mutation of leucine to alanine at residue 208 has **significantly inhibited growth** in the absence of IPTG (Figure 5). This evidence suggests that **L208 is an essential residue for DnaI function** and subsequently DNA replication in *B. subtilis,* potentially due to its role in binding single-stranded DNA.

FUTURE WORK

Purify the DnaI (L208A) mutant and determine if it has lost single-strand DNA binding activity.

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REFERENCES

[1] Arias Palamo et al., 2019, Physical Basis for the Loading of a Bacterial Replicative Helicase onto DNA. Molecular cell 74, 1-12
[2] Figures created using BioRender under a paid subscription.
[3] Charles Winterhalter. Unpublished