# Study of the behaviour of *soj* mutants and discovery of new *soj* mutants with a significant trapping defect

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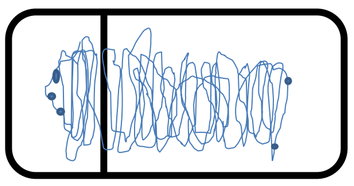
## Introduction

One fundamental question Science has tried to answer is the problem of how a single cell divides asymmetrically to produce different daughter cells during development. The complex nature of mammalian systems makes it impossible to directly study their development however, studying the process in simple organisms can give us insight into how it works (Errington, 2010).

During sporulation, *Bacillus subtilis* divides asymmetrically in the presence of appropriate signals (shown in the diagram below) hence making it a suitable model to study asymmetric division.



The main focus of my project was on *soj*, a protein involved in the segregation of the OriC into the prespore compartment. Two of the previously discovered *soj* mutants, G12V (GJS073) and R189A (GJS126) have been found to have a wild type phenotype while K16A (GJS127) and D40A (GJS101) have a trapping defect

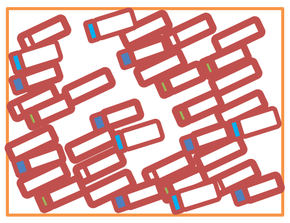
## Aims

1. To study the behaviour of 4 *soj* mutants (G12V, R189A, K16A and D40A) in a trapping assay and determine which species can overrule the effect of the other when 2 mutants with opposite effects are combined in the assay.
2. To determine if there are any *soj* mutants with a very strong defect in trapping OriC

## Materials and methods

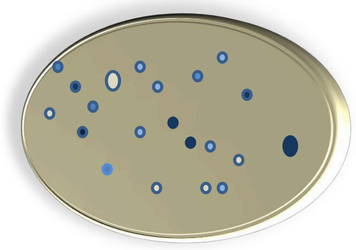
For the first part of the project:

- Strains containing the 2 *soj* alleles were constructed. One was transformed into the native locus and the other at the ectopis amyE locus.
- Flourescence microscopy was used to quantify (a shown in the diagram below) trapping of the OriC-proximal and OriC –distal chromosomal markers in the prespore compartment of the strain using the wild-type as the control as well as an empty vector control (GJS128).



For the second part of the project:

- A low fidelity DNA polymerase was used to obtain random *soj* mutants in a PCR.
- The mutants were then transformed into a background that was designed to give blue colonies in the presence of a trapping defect as shown in the diagram.



- The very blue colonies were sent for sequencing since the have a strong defect in positioning the OriC in the prespore compartment.

## Results

### Comparison of opposing *soj* mutants in a trapping assay

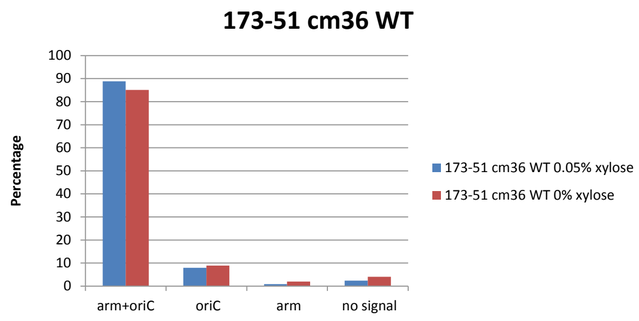



Fig 1.Graph showing the percentage distribution of the arm and OriC in the prespore for the wild-type (control)

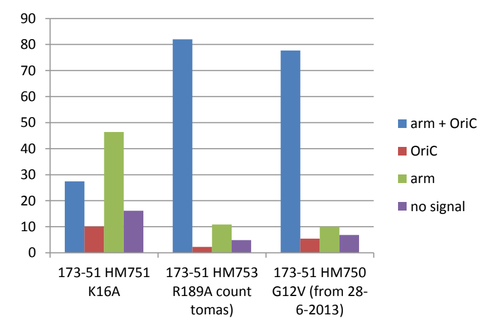



Fig 2. Graph showing the percentage distribution of the OriC and arm in the prespore for the different Soj mutants (except D40A)

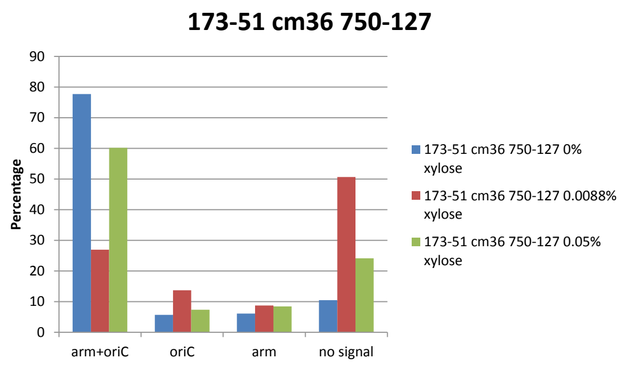



Fig 3. Graph showing the percentage distribution of the OriC and arm in the prespore of HM750 GJS127 strain (G12V in native locus, K16A in AmyE locus).

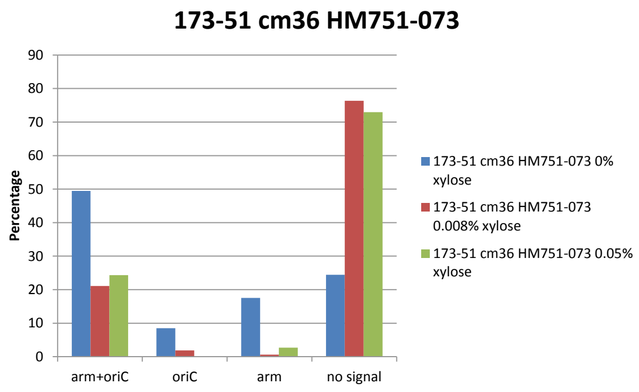



Fig 4. Graph showing the percentage distribution of the OriC and arm in the prespore of HM751 GJS073 strain (K16A in native locus, G12V in AmyE locus).

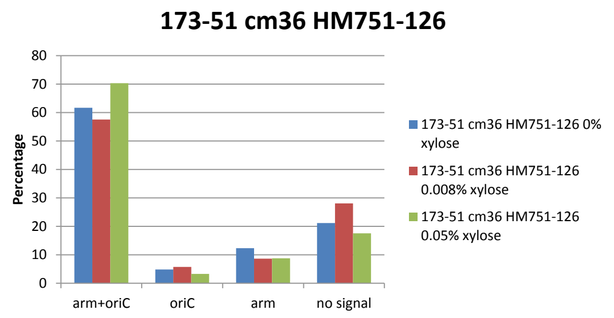



Fig 5.Graph showing the percentage distribution of the OriC and arm in the prespore of HM751 GJS126 strain (K16A in native locus, R189A in AmyE locus).

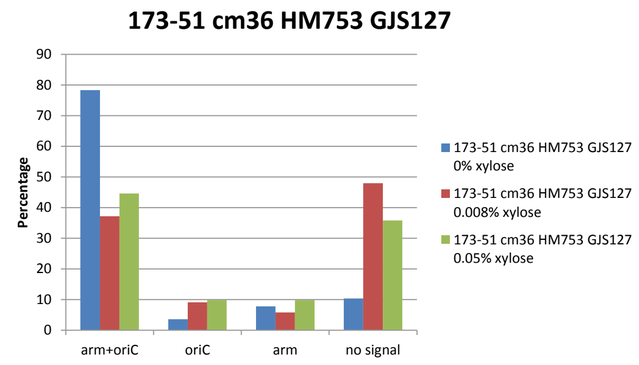



Fig 6. Graph showing the percentage distribution of the OriC and arm in the prespore of HM753 GJS127 strain (R189A in native locus, K16A in AmyE locus).

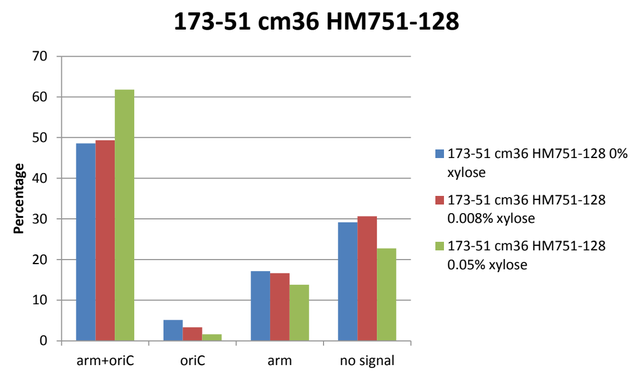



Fig 7. Graph showing the percentage distribution of the OriC and arm in the prespore of HM751 GJS128 strain (K16A in native locus, empty vector control in AmyE locus).

## •Conclusion

•K16A has the strongest defect and can abolish 'wt-like' trapping frequencies of R189A (non-DNA binding mutant) and G12V (ATP-bound monomer form).
•K16A had a greater effect in the ectopic locus where it was expressed in a xylose-dependent promoter than in the native locus.
•Results with D40A were very difficult to analyse due to weird effects on cell morphology therefore no tangible conclusions can be made from it.
•V14A is located in the ATP binding site (active site) of Soj which is probably why it is so defective.

## Acknowledgements

I would like to specially thank Prof Errington, Dr Kloosterman, Dr Ling Juan Wu and all the staff at CBCB for facilitating this project. I also acknowledge the support of the Society for General Microbiology for granting me the Harry Smith Vacation Studentship.

## References

•Errington, J. (2010). From spores to antibiotics via the cell cycle. *Microbiology*. 156 (1), 1-13.