The effect of Spo0J spreading on SMC protein complex

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Introduction

Structural Maintenance of Chromsomes (SMC) protein complexes are present in diverse organisms ranging from bacteria to man. These protein complexes have an array of functions including chromosome compaction, segregation and DNA repair. Proposedly, SMC proteins condense newly synthesised regions and draw them away from replication machinery. [1]

SpoOJ proteins use different mechanisms to regulate chromosome segregation and also sporulation (bacteria switching to a vegetative state in case of adverse envinronment). They bind to DNA near *oriC* at specific binding sites (ParS) and then laterally spreads.[2] It has been shown that these proteins are important for positioning the origins of replication and influencing the initiation of replication.[6] The importance of this gene can be seen in null mutants that produce 1-2% anucleate cells – 100 times more than present in wild type cells.[7]

Resent research [2] has shown interaction between these proteins and the aim of this project was to test whether:

SMC protein complex recruitement at *oriC* regions requires lateral spreading of Spo0J proteins (Fig 1.)

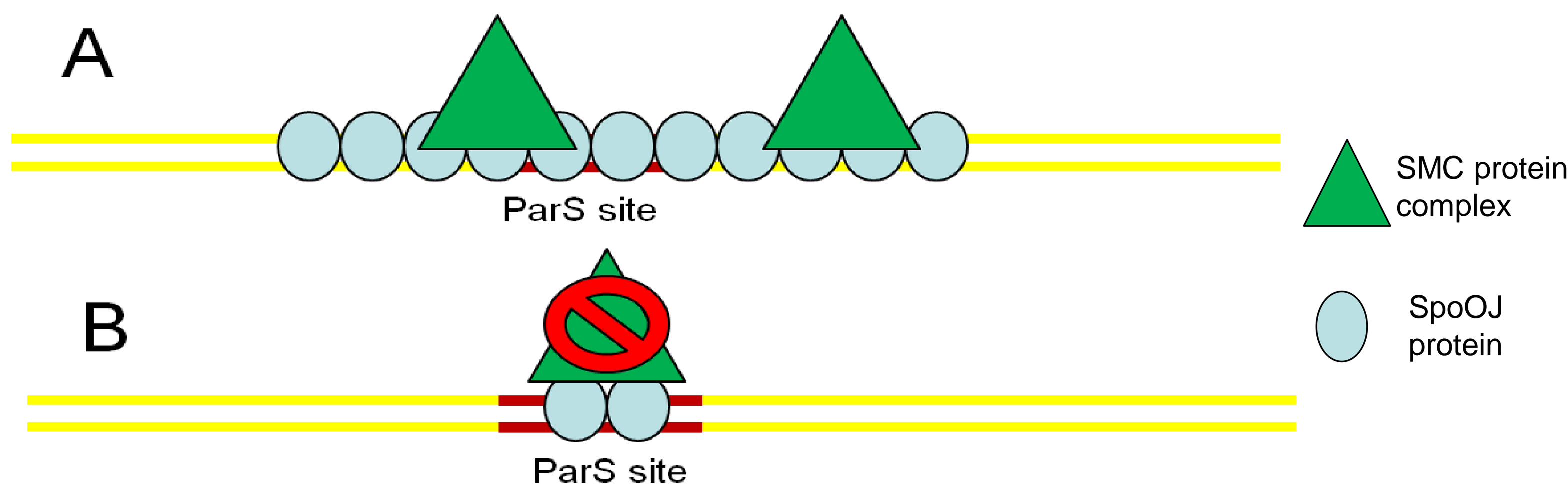

Fig 1. Part A – A simplified model of SpoOJ (blue ovals) and SMC protein complex (green traingles) interaction proposed by Gruber and Errington, 2009. SpoOJ (also called ParB) proteins are binding the ParS site and laterally spreading, while SMC protein complex uses this conformation to attach itself to the chromosome. Part B – The results of this project suggests that SpoOJ proteins binding to a parS site is not enough for SMC protein complex to interact with it and the chromosome.

To understand this phenomenon mutants with defective SMC and SpoOJ protein interaction from the study of Gruber and Errington, (2009) were analysed (Fig 2). Another aim of this project:

Identify more *spo0J* mutations that specificially affect interaction of Spo0J and SMC proteins

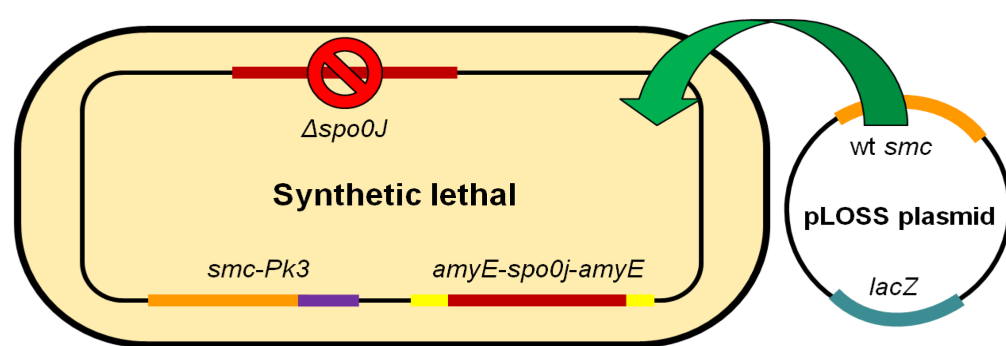

Fig 2. Screening of the mutant strains. *smc-Pk3* construct together with mutated *spo0J* in *amyE* locus creates a synthetic lethal phenotype. Wt *spo0J* is deleted. The phenotype is rescued by pLOSS plasmid containg *wt smc* and seen as blue colonies when grown on X-Gal plates due to *lacZ*. Second screen on normal plates was used to ensure that sporulation is normal.[2]

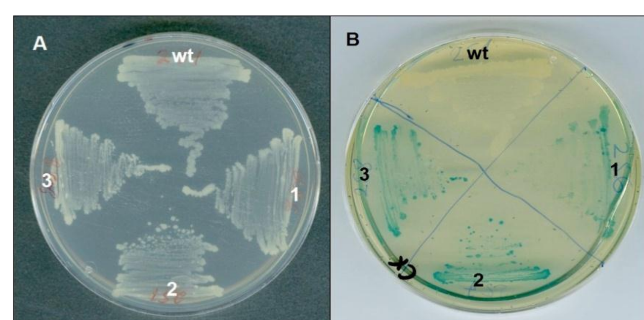

Figure 3. Strains 1(BSG256), 2(BGM257), 3 (BGM258) with mutated *spo0J*.[2] Part A shows the comparison of sporulation activity of strains 1,2 and 3 with wild type (wt). Part B demonstrate the synthetic lethal effects of *spo0J* mutation as the strain is keeping the plasmid to ensure its viability.

References

1. Graumann PL, Knust T. (2009) *Chromosome Res.* **17,** 265-275.
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5. Kusiak M. et al. (2011) *J Bacteriol.* **193,** 3342-3355.
6. Lee PS, et al. (2003) *J Bacteriol.* **185,** 1326- 1337.
7. Ireton K, et al. (1994) *J Bacteriol.* **176,** 5320-5329.

Results

Analysis of *spo0J* mutations on the protein structure

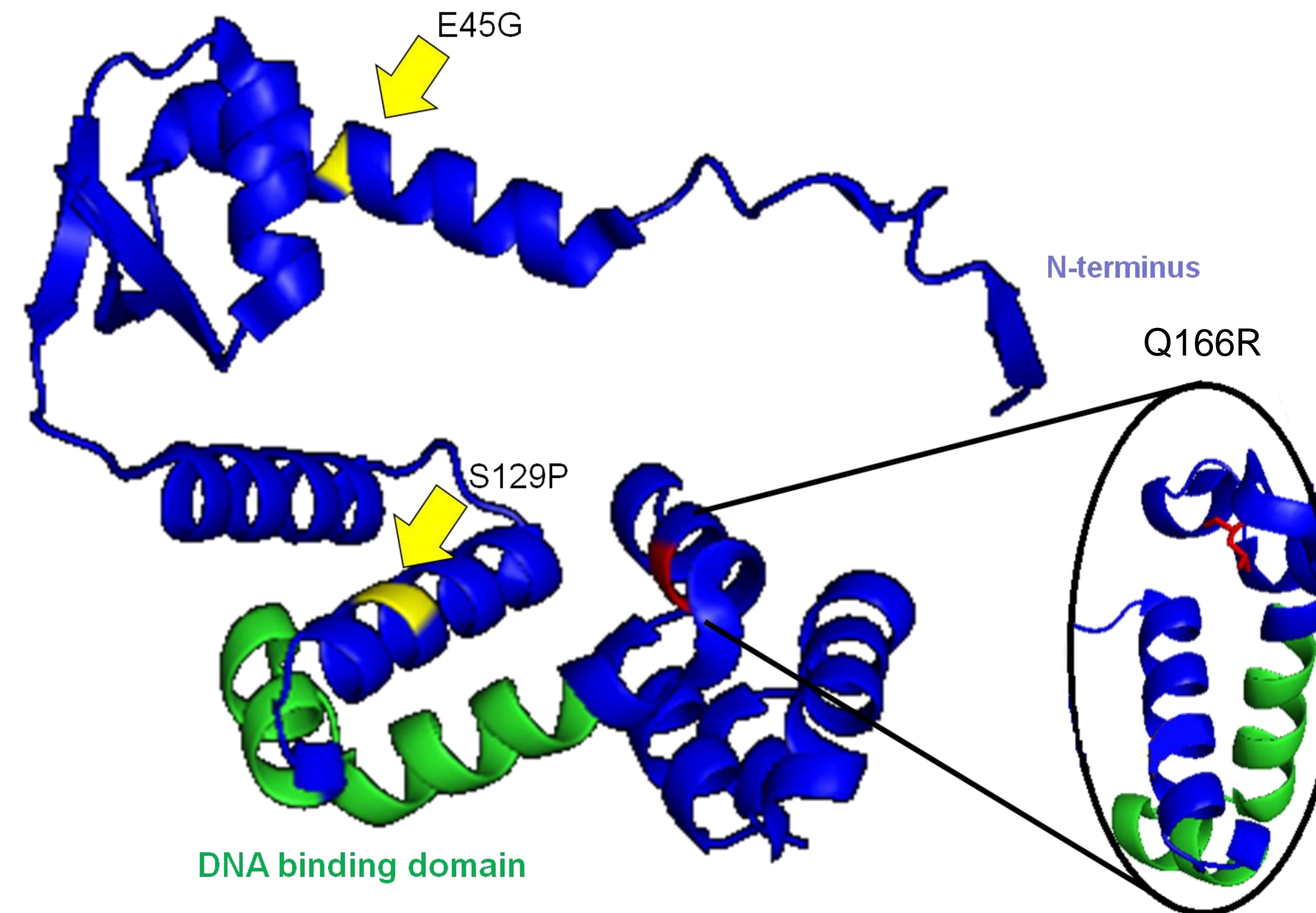

Figure 4. *Spo0J* mutations present in *B.subtilis* strains created in this project (L3, L4) were mapped on *Th.thermaphilus* Spo0J protein crystal structure.[3] *B.subtilis* and *Th.thermaphilus* protein sequences were aligned using Clustal and corresponding mutated amino acids were mapped. As this crystal structure does not contain C terminus, amino acids present in this part of the protein could not be shown.

Spo0J binding efficiency determined by ChIP and qPCR

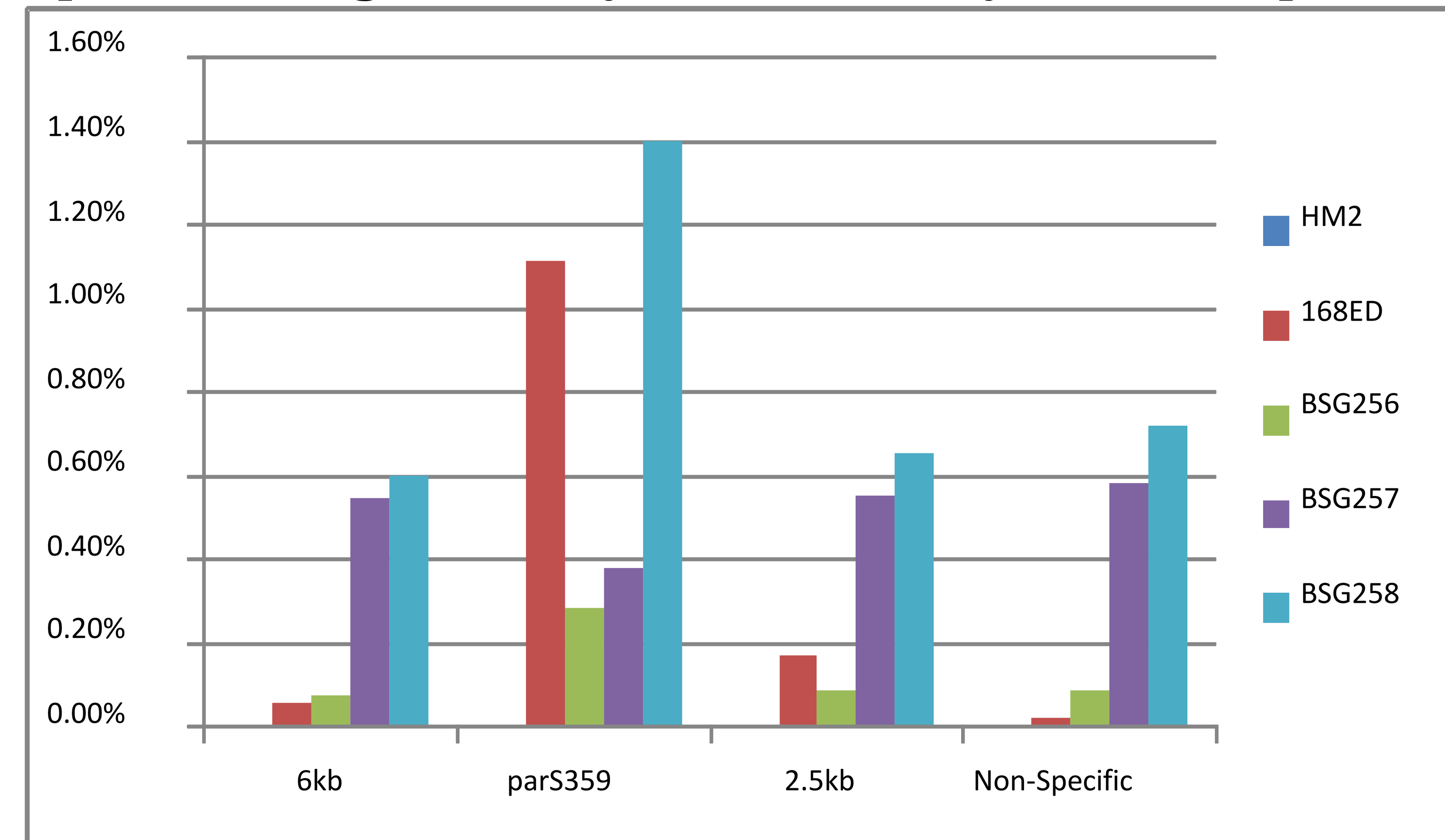

Figure 5. SpoOJ binding and spreading was tested by chromatin immunoprecipitation (ChIP) of SpoOJ bound DNA complexes followed by qPCR. Primers selected at ParS site and on both sides of it with gradual increase of distance – 2.5 and 6 kb, as well as at the opposite side of the chromosome to check for non specific binding. Mutant strains with defective interaction (BSG 256-8) were compared with wild-type (168 ED) and *ΔSpoOJ*(HM2) strains. Quantitative PCR results are shown as percentage of the input and are the means of triplicates.

BSG256 does not seem to bind so well when compared to wildtype, while the other two strains (BSG257 and BSG258) show stronger but non specific signal and further tests are necessary to determine the phenotype.

Conserved residues in mutant *spo0J*

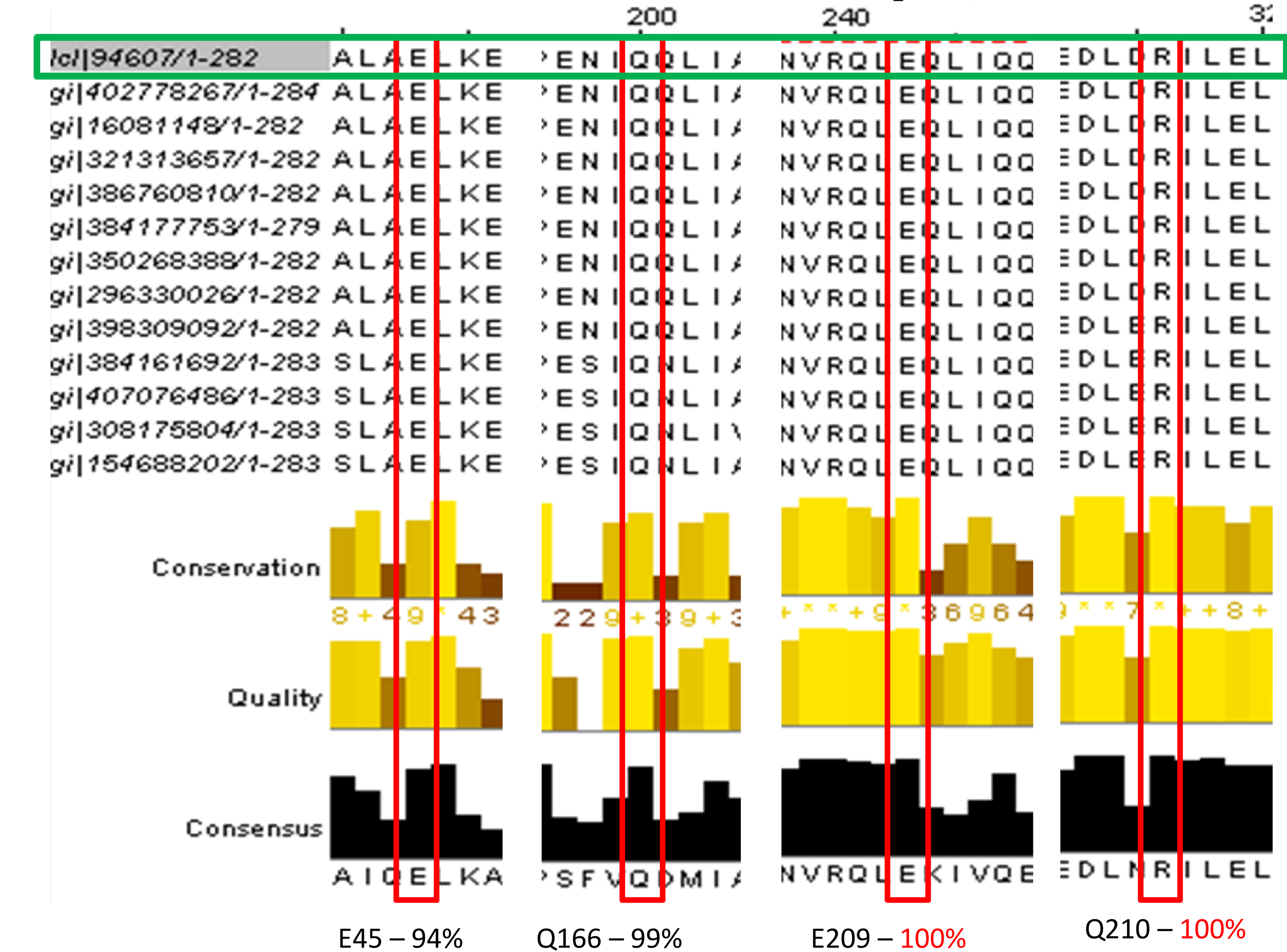

Figure 6. Some of the mutations occurring in strains L3, L4 and L6 appeared to be strongly (94-100%) conserved when 100 sequences within 20 species of bacteria – parts of 12 sequences (*B. subtilis* in green) are shown in this figure. This analysis was done using BLAST to align the SpoOJ protein sequence and Jalview 2.7 to analyse the data.

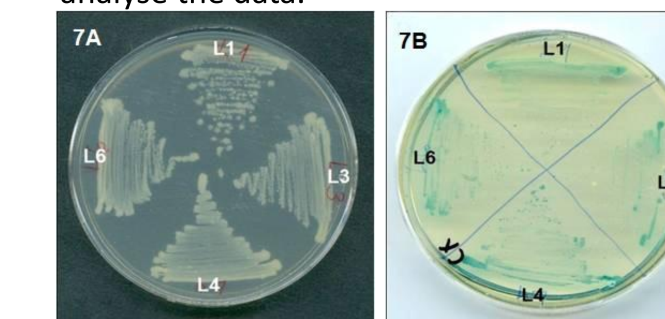

Fig 7. Strains L1, L3, L4 and L6 were produced during this project. *Spo0J* gene was mutagenised by error-prone PCR and inserted in *amyE* locus by bacterial transformation. The screening method applied and genotype are described in Fig 2. 7A shows the effiency of sporulation, 7B – synthetic lethal phenotype.

Conclusions

- Spo0J proteins seem to bind DNA in strains with defective Spo0J protein and SMC protein complex interaction but the lateral spreading is heavily affected, sugesting that the spreading is required for normal SMC protein complex loading on chromosome.
- Non – specific Spo0J binding was thought to be one of the possible explanations for the synthetic lethal phenotype, yet this project did not produce any proof for that and more work on this problem is required.
- Strains with mutated *spo0J* and defective interaction with SMC protein complex produced in this project contained mutations close to DNA binding domain, C terminus – required for dimerization[4] and N terminus (oligomerization)[5]. Some of mutated residues were strongly (94-100%) conserved.

Future work

- Three of the mutant strains (L3, L4 and L6) possess more than one mutation. Therefore, these mutations should be looked at separately to determine which ones are causing the phenotype.
- Strains L1, L3, L4 and L6 should be analysed by ChIP and qPCR to see how binding and lateral spreading of SpoOJ proteins was affected.
- Non-specific SpoOj binding hypothesis could be tested more throughoutly by qPCR with more primers at non-ParS sites over *B. subtilis* genome.