Optimizing a mutation system for *Bacillus subtilis*

Claire McGahan[1]*, 08052951, BSc Human Genetics, claire.mcgahan@newcastle.ac.uk
Wendy Smith[1,2], Neil Wipat[1,2]
[1]Institute for Cell and Molecular Biosciences, [2]School of Computing Science, Newcastle University, Newcastle upon Tyne UK NE2 4HH

Introduction

Bacillus subtilis is a non-pathogenic, gram-positive soil bacterium. *B. subtilis* strain 168 has been intensively studied as a model for Gram-positive bacterial physiology, due to its susceptibility to genetic manipulation and accessibility of a well-annotated genome sequence. However, despite the genetic amenability of *B. subtilis* there are still a number of limitations in strategies for generating chromosomal mutations.

The aim of my project is to optimise and test a highly effective allele-replacement mutagenesis system currently used in *Streptococcus pyogenes* for constructing 'clean' chromosomal mutations, for use in *B. subtilis*.

Construction of recombinant plasmid pCMG1

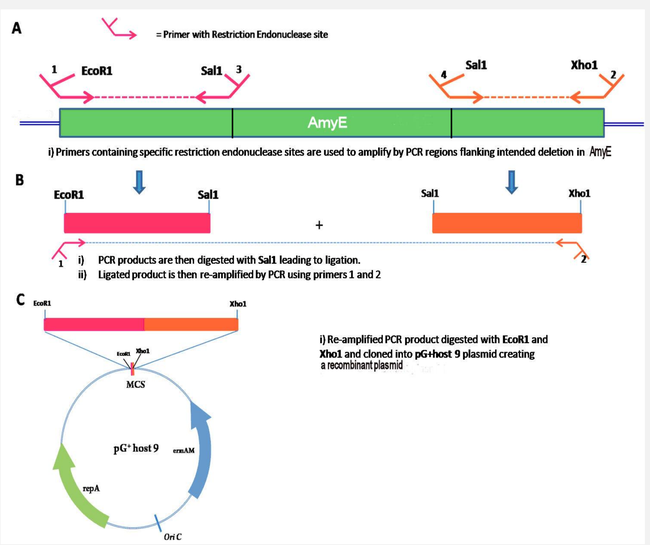

Figure 1: Construction of recombinant plasmid pCMG1. This was constructed to facilitate production of an in-frame deletion of *amyE,* using 250 bp of sequence upstream and downstream of the *amyE* coding region. The desired deletion could then be introduced into the chromosome *via* allele replacement mutagenesis as summarized in **Fig 2**.

Construction of an *amyE* deletion mutation

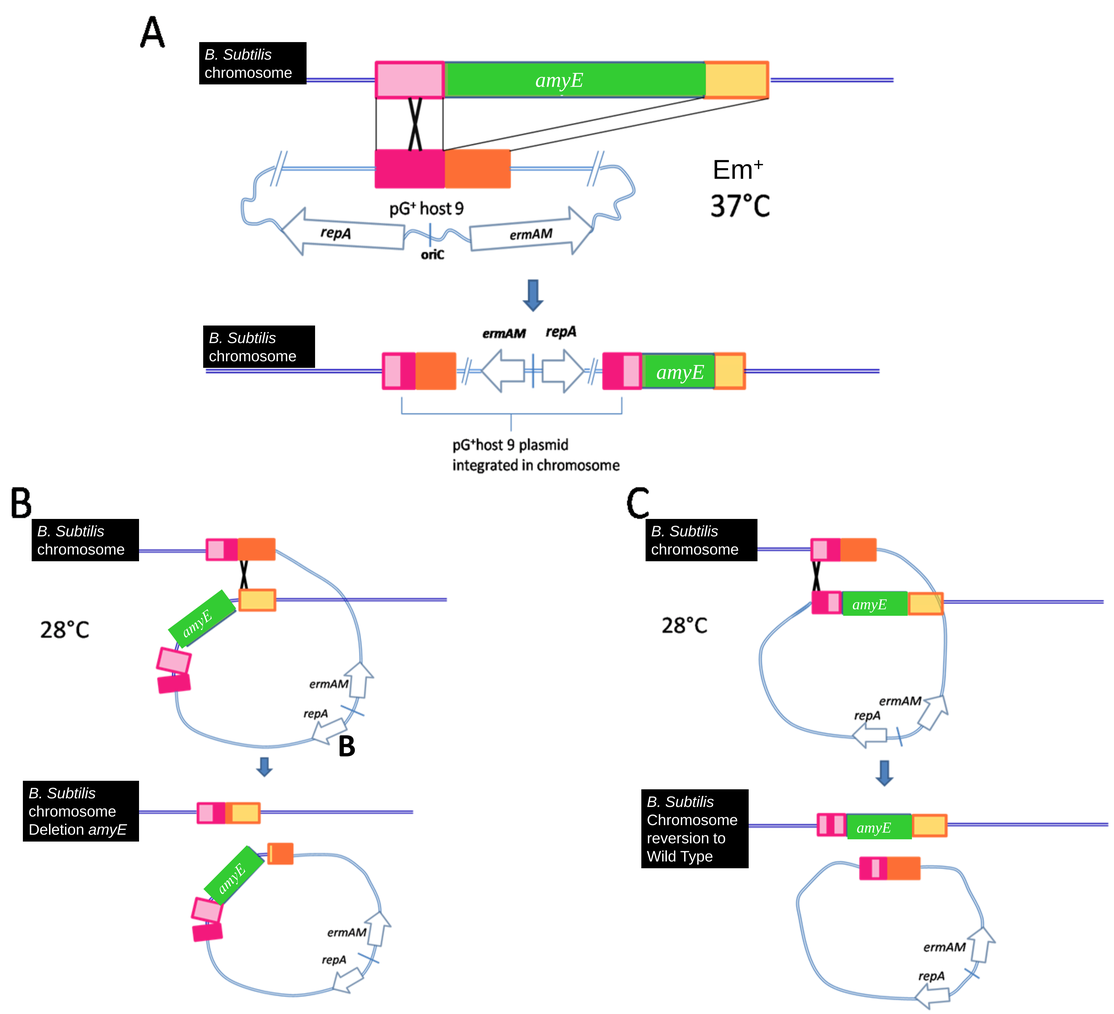

Figure 2: Allele-replacement mutagenesis

This temperature-sensitive pG+Host 9 allele-replacement system involves a well documented two-step protocol (Smith *et al*, 2010), where direct selection at the non-permissive temperature results in the integrated plasmid flanked by duplicated copies of the target sequence. A temperature shift then stimulates plasmid excision at a sufficient rate to enable potential mutants to be identified by screening initially for loss of the selective marker, followed by PCR screening of sensitive colonies.

We are focusing on a deletion of the *amyE* gene where wild-type and mutant colonies can be distinguished directly on starch plates.

Transformants in *B. subtilis* 168 were grown at either 26, 28 or 30°C (permissive temperature) in LB + 5ug/ml Em to mid-log phase then shifted to 37°C (non-permissive temperature) overnight to inhibit replication of the pGh9Δ*amyE* plasmid and allowing growth of cells with a single cross-over (plasmid integration onto the chromosome).

Results

Confirmation of recombinant plasmid

E. coli DH5 α transformants containing the recombinant plasmid were selected for on Em+ plates and identified by agarose gel electrophoresis after digestion with EcoRI and XhoI .

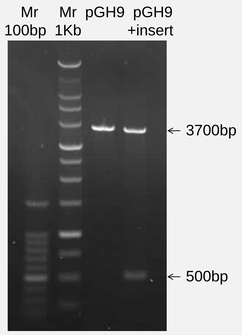

Figure 3: Confirmation of pCMG1

Optimising conditions for a single crossover event in B. subtilis 168

Single crossover integration frequencies were determined for three different permissive temperatures.

Table 1: Integration frequencies

Temperature	Integration Frequency
26°C	3.54×10^{-7}
28°C	4.16×10^{-7}
30°C	3.71×10^{-7}

Conclusions

Initially several integrants resistant to erythromycin were obtained due to a single cross over event having occurred (Fig. 2A). The fact that the first stage of the protocols worked is encouraging and suggests that it may be possible for this method to work in *B. subtilis* with a few minor alterations.

References

Smith, W.D; Pointon, J.A; Abbot, E; Kang, H.J; Baker, E.N; Hirst, B.H; Wilson, J.A; Banfield, M.J; Kehoe, M.A. (2010) Roles of minor pilin subunits Spy0125 and Spy0130 in the serotype M1 *Streptococcus pyogenes* strain SF370. J Bacteriol 192:4651-9