Mecillinam resistance mediated by overproduction of the endopeptidase MepS

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Introduction

Peptidoglycan (PG) is a mesh-like layer essential in creating a protective barrier in bacterial envelopes. PG consists of glycan chains made of repeating units of N-Acetylglucosamine (Glc*NAc*) and N-Acetylmuramic acid (Mur*NAc*). Glycan chains in *Escherichia coli* are commonly connected between the 4th amino acid and the 3rd amino acid of adjacent peptide chains stemming from Mur*NAc*.
Mecillinam is a antibiotic that inhibits penicillin binding protein 2 (PBP2) preventing cross-linking of glycan chains. Therefore, Mecillinam-treated *E. coli* are unable to divide and eventually lyse as PBP2 is required to elongate PG.
Mecillinam resistance is induced under MepS and FtsZ overexpression[1]. MepS is a PG hydrolase, meaning it breaks bonds in PG to allow insertion of nascent PG. FtsZ is a division protein
We deleted the genes of the enzymes we thought could be involved in the mechanism inducing resistance under MepS upregulation.

So the following *E. Coli* strains were used:

- Wild type (**WT**) strain as a control
- WT with deletion in PG synthases (**PBP1A-, PBP1B-**). These synthesise PG by creating crosslinks.
- WT with deletion in carboxypeptidase (**PBP5-**). PBP5 cleaves peptide stems of nascent PG to allow addition to existing PG.
- WT with deletion in PG transpeptidases (**LdtD-, LdtDEF-, 6LDT-**). L,D-transpeptidases (LDT's) form a different crosslink that is suggested to increase specific antibiotic resistance. There are 6 LTD's present in *E. coli* named LdtA to F.

Procedure

Each strain upregulating FtsZ was transformed with either plasmid:
- pBAD33 containing the gene for MepS to overexpress MepS
OR
- pBAD33 with no gene to overexpress to act as a control to show MepS overproduction is responsible for any differences seen.

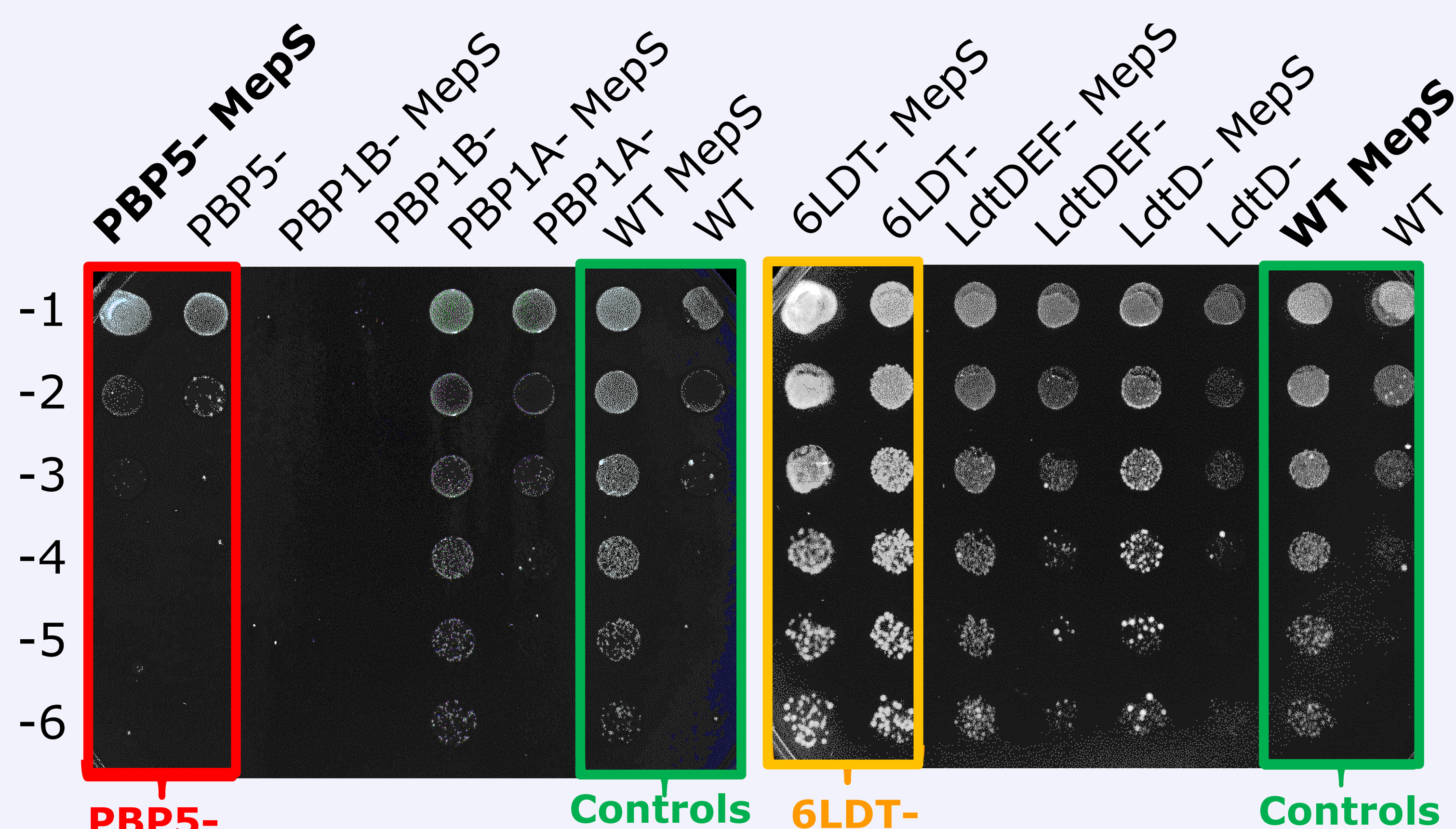
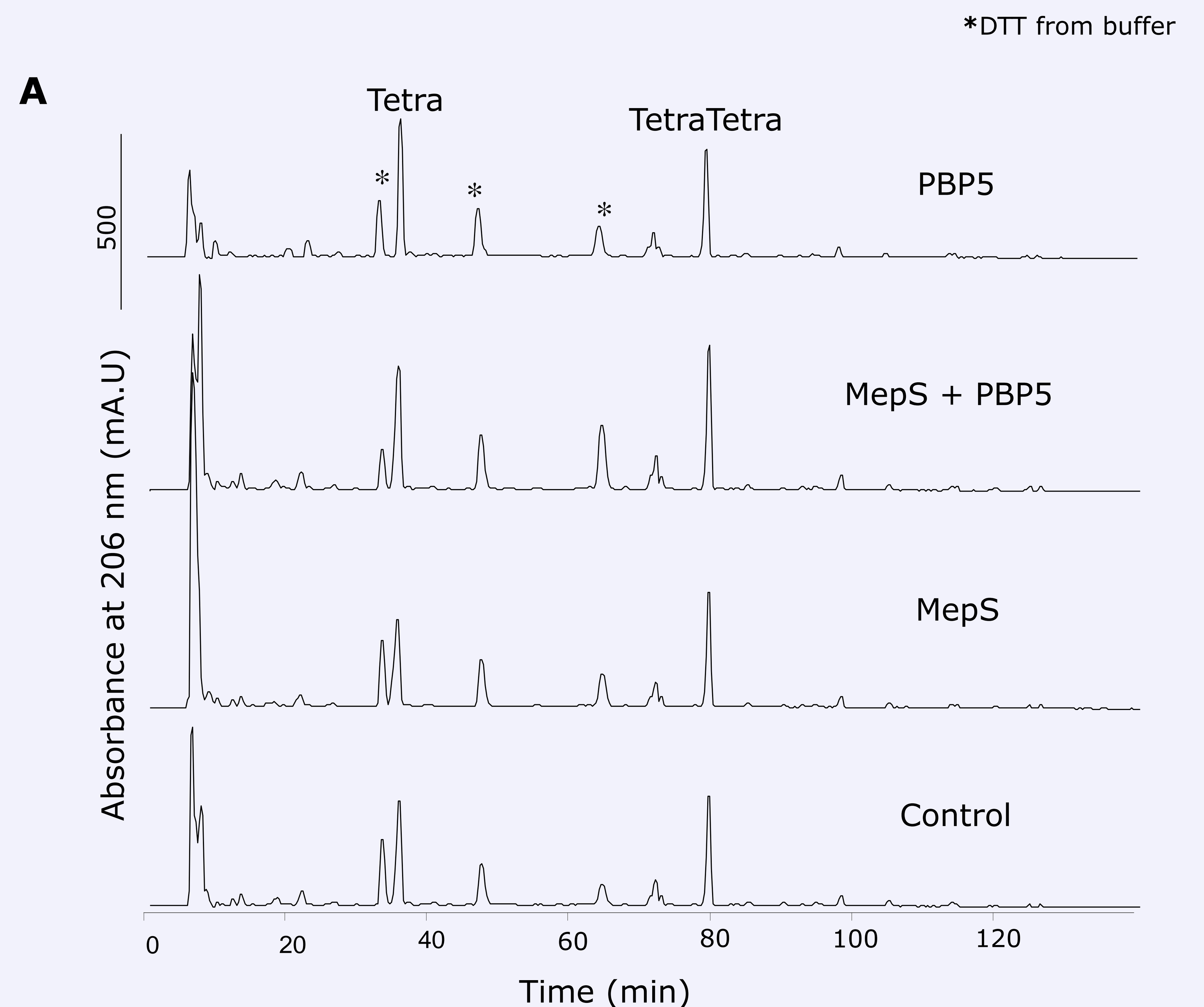


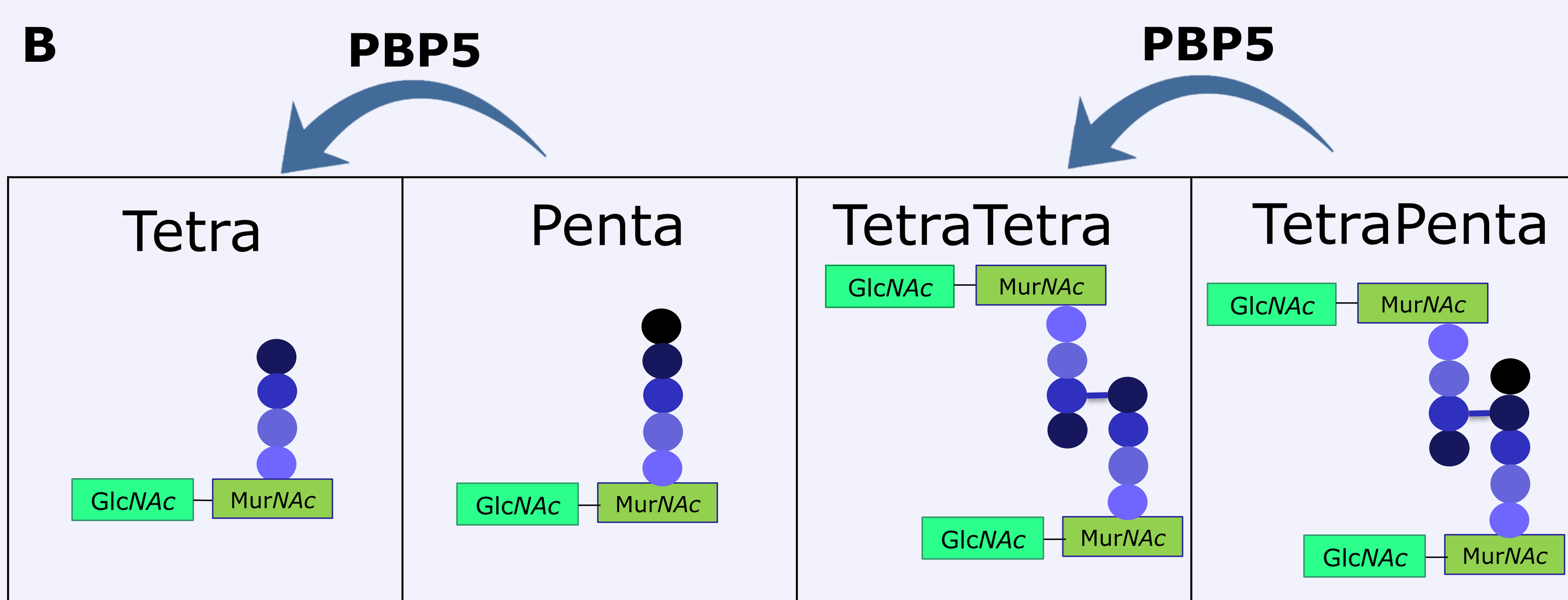
Fig. 1. *E. coli* with a deletion in PBP5 is less resistant to mecillinam compared to the WT when MepS is overproduced. *E. coli* grown at 30ºC with antibiotics to keep the plasmids. Normalised to an optical density of 0.1 at 600nm, serially diluted by 1:10 and stamped onto solid agar containing mecillinam and a sugar to induce pBAD33 to over-express MepS. Mecillinam only plates showed no difference in growth when overexpressing MepS or not, showing differences are resistance are purely down to MepS overexpression.

Main results from screening:

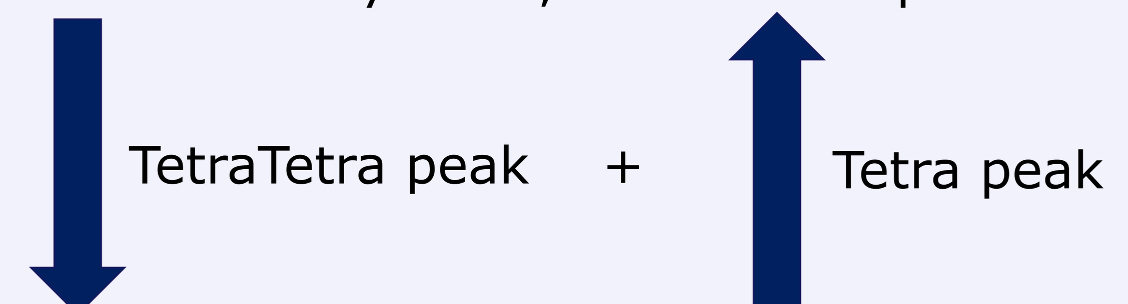
PBP5- MepS overproduction doesn't increase resistance as much when compared to WT. Therefore, PBP5 may be involved in the resistance mechanism. Investigated if PBP5 directly activates MepS in Fig.2. If so, it would explain PBP5's role in inducing resistance.

6LDT- Deletion in all 6 LD-transpeptidases lead to an increase in resistance compared to controls, so may have induced a stress response leading to mecillinam resistance.





If MepS was activated by PBP5, we would expect:



when compared to conditions where one/neither are present, but this is not seen

Fig. 2. PBP5 does not directly activate MepS.
(A) Profiles of purified WT PG of *E. coli* after adding PBP5 and MepS together, separately or with neither (Control). After adding appropriate salt, water, buffer, PG and the required proteins for each condition, the samples were incubated whilst shaking for 5 hours. Peptidoglycan fragments were obtained from digestion with cellosyl. Samples were then reduced with DDT and analysed using High-performance Liquid Chromatography to detect these fragments.
If MepS is active we expect to see an increase in the Tetra peak and a decrease in TetraTetra peak when compared to the controls. This was not seen; these peaks remain the same in each condition. Therefore MepS is not directly activated by PBP5.
(B) Structures of PG fragments labelled on peaks in Fig.2A. and reactions active PBP5 and MepS undergo.

Conclusions and further study

- There is an indirect link between PBP5 and MepS under MepS overproduction that induces mecillinam resistance. How is yet to be further investigated.
- Further study may include why the deletion of all 6 LDT's induces a particular stress response.
- See the effects on mecillinam resistance during MepS overproduction in other conditions

References
1. Lai *et al.* The mecillinam resistome reveals a role for peptidoglycan endopeptidases in stimulating cell wall synthesis in *Escherichia coli*. 2017. PLoS Genetics **13** (7)): e1006934