

## Introduction:

Biological function of penicillin binding protein 3 (PBP3) in *Bacillus subtilis* (encoded by the *pbpC* gene) showed potential similarities to the penicillin resistance determinant *mecA* (PBP2a) of *Staphylococcus aureus* at the amino acid sequence level. Here we find that the loss of this PBP increases the sensitivity of *Bacillus* to specific  $\beta$ -lactams. Whereas the literature cites *sigM* as being responsible for resistance to penicillins.

## Aim:

To evaluate PBP3 contribution to the intrinsic resistance of *B. subtilis* to  $\beta$ -lactams resistance

## Methods:

The effect of *pbpC* and/or *sigM* knockouts either singly or in combination on  $\beta$ -lactams resistance were determined:

- Strains of the wild type (168), *pbpC* knockout, *sigM* knockout and double knockout were overnight grown
- Each strain treated with different antibiotics (penicillin G and oxacillin). Samples were taken at specific time points following antibiotic treatment.
- Optical density of growth was tested for each sample. Colony forming units (CFU) were also monitored (Data not shown)
- Same procedure was carried out to test the effect of the antibiotics on plates

## Results:

- *pbpC* knockout and double knockout (*pbpC*, *SigM*) are more sensitive to oxacillin than the other strains (Fig.1)
- *SigM* knockout and double knockout (*pbpC*, *SigM*) strains are more sensitive to penicillin G than the other strains (Fig.2)

## References:

- Luo, Y. and Helmann, J. D., 2012. Analysis of the role of *Bacillus subtilis*  $\sigma^M$  in  $\beta$ -lactam resistance reveals an essential role for c-di-AMP in peptidoglycan homeostasis. *Molecular Microbiology*, 83(3), 623-639.
- Thackray, P. D. and Moir, A., 2003. SigM, an Extracytoplasmic Function Sigma Factor of *Bacillus subtilis*, Is Activated in Response to Cell Wall Antibiotics, Ethanol, Heat, Acid, and Superoxide Stress. *Journal of Bacteriology*, 185(12), 3491-3498.
- Xu, M., Emmins, R., Errington, J. and Daniel, R. A., Catalytic Redundancy of Cell Division Penicillin-Binding Proteins in *Bacillus subtilis* (in preparation).

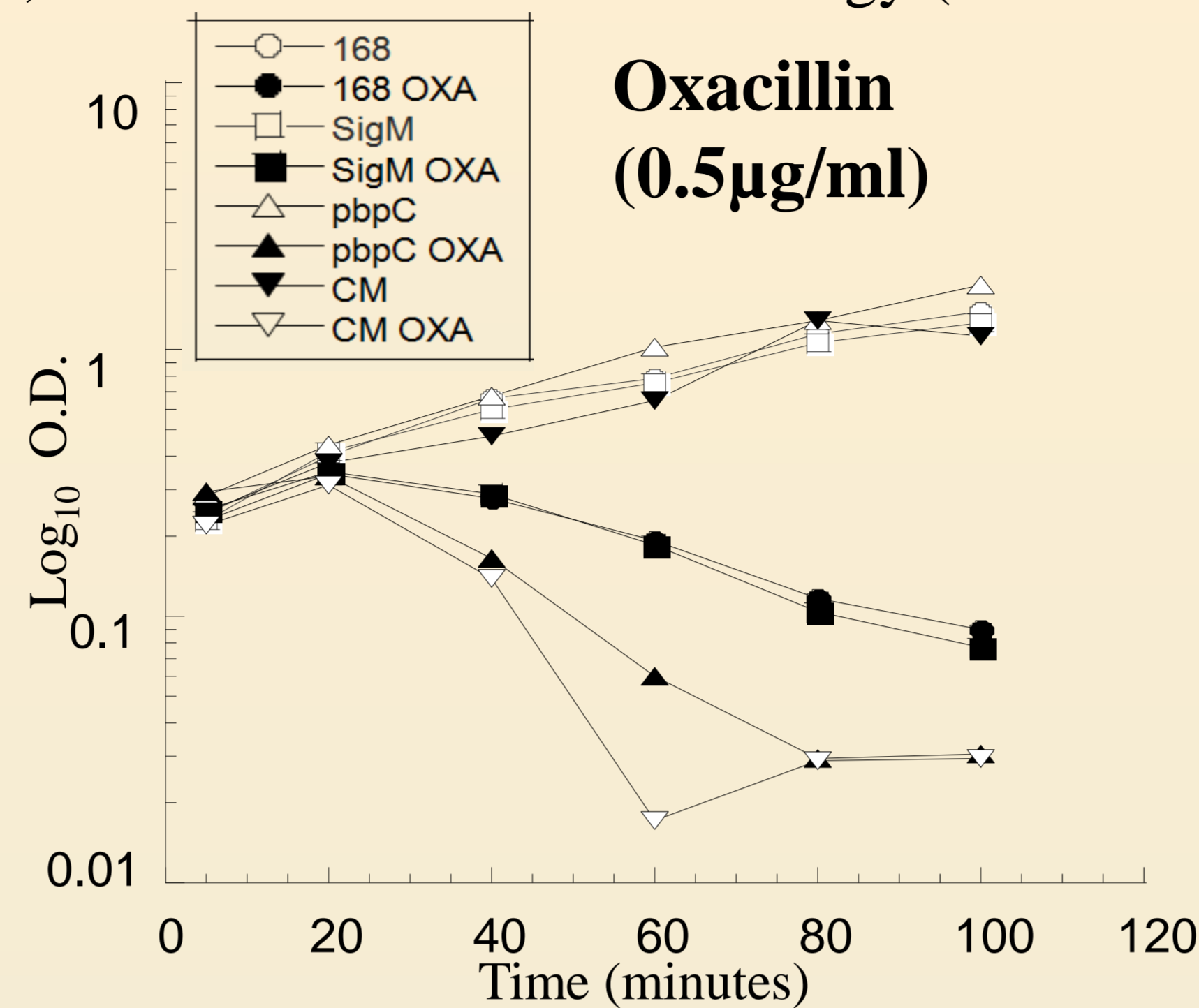


Figure 1: The effect of Oxacillin

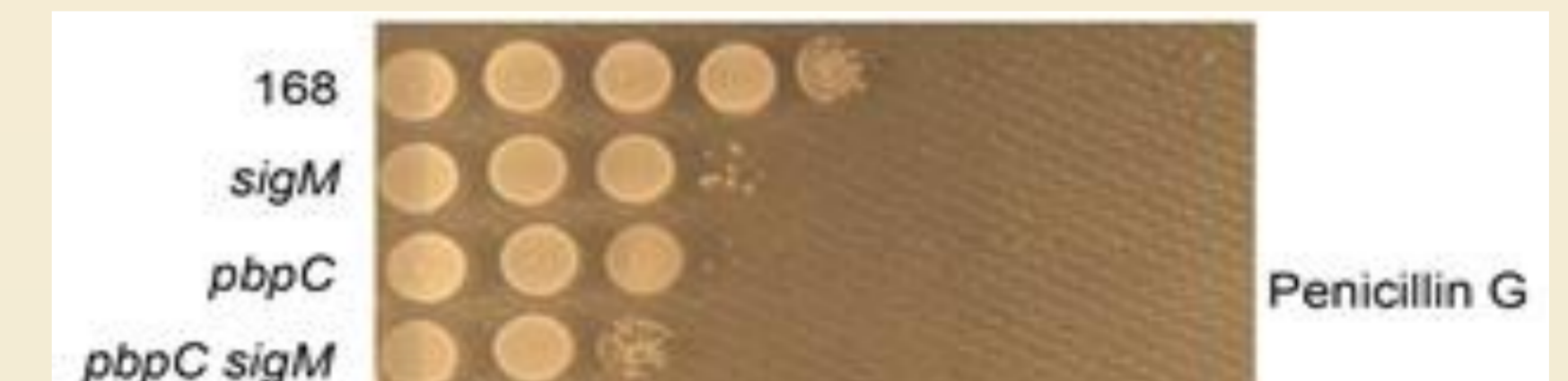
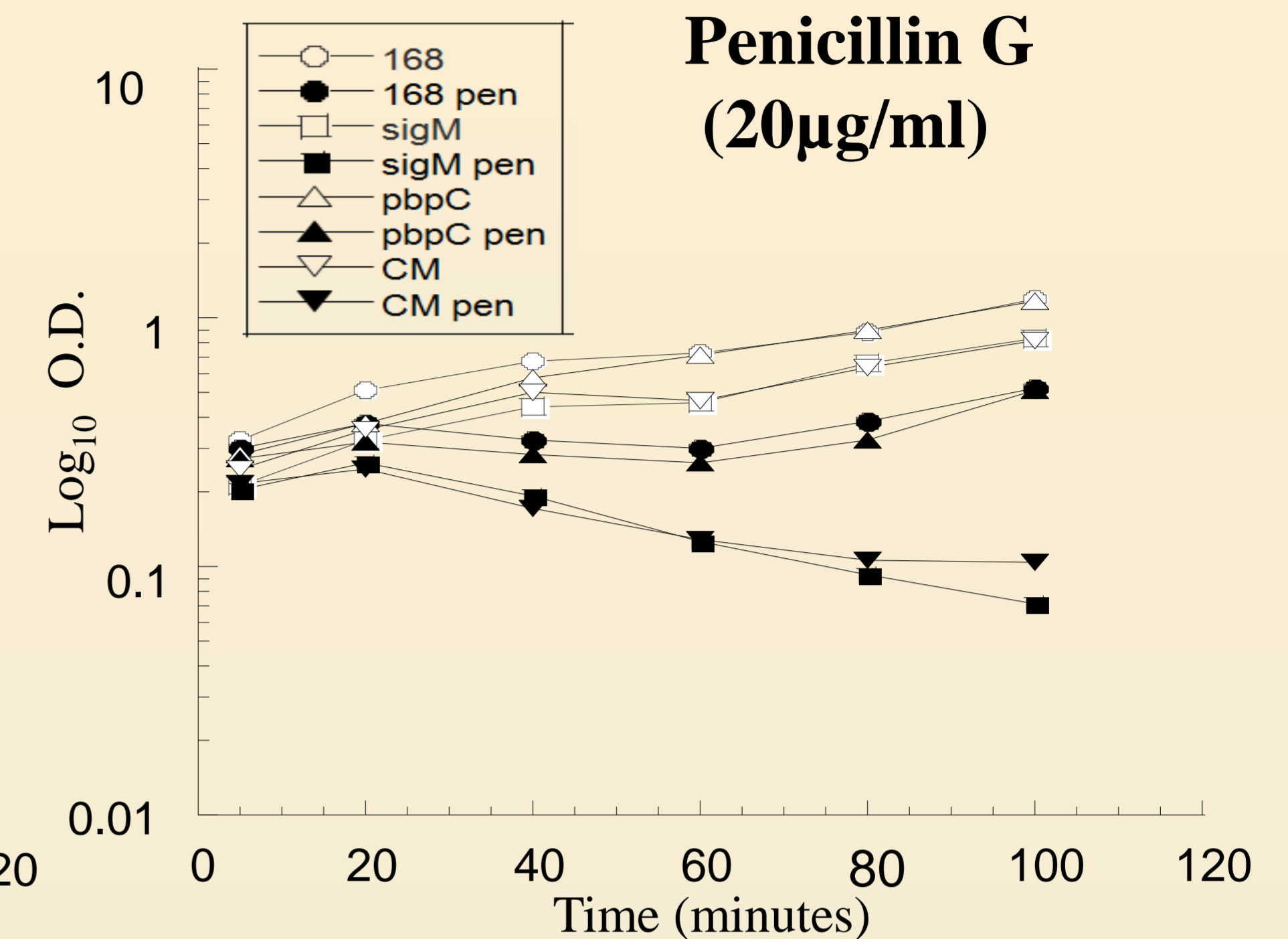


Figure 2: The effect of PenG



Figure 3: Nutrient agar plate (no antibiotic)

## Discussion and conclusions:

- Oxacillin blocks the action of some cell division PBPs. PBP3 can overcome this problem. This explains why strains lacking *pbpC* are more sensitive to oxacillin than the other strains.
- Penicillin G blocks the action of all PBPs except PBP3. This explains why penicillin G has similar sensitivity effect on *pbpC* knockout and wild type strains. The bacteria become more sensitive to penicillin G when they lose SigM factor as it seems to have a role in the adaptation to stress caused by the antibiotic rather than providing resistance.