Dissecting the molecular mechanisms of MRSA; a threat to food security.

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BBSRC Doctoral Training Partnerships

MIBTP Midlands Integrative Biosciences Training Partnership

WARWICK
INTEGRATE ANTIMICROBIAL RESISTANCE

Background

Staphylococcus aureus is a major cause of **agricultural livestock mastitis**, with the threat posed magnified by the acquisition of the mecA gene, a resistant determinant causing high level **β-lactam antibiotic resistance**. MecA encodes **penicillin binding protein 2a** (PBP-2a), which has a low affinity for β-lactams allowing peptidoglycan synthesis to occur in otherwise lethal concentrations of β-lactams. The FemX, FemA and FemB ligases (Fig. 1) also play a critical role in β-lactam resistance, providing PBP-2a with the peptidoglycan precursor Lipid-II (Lys) Gly_5. The overall aim of this study is to investigate the role of the FemXAB ligases and PBP-2a in methicillin resistant *S. aureus*.

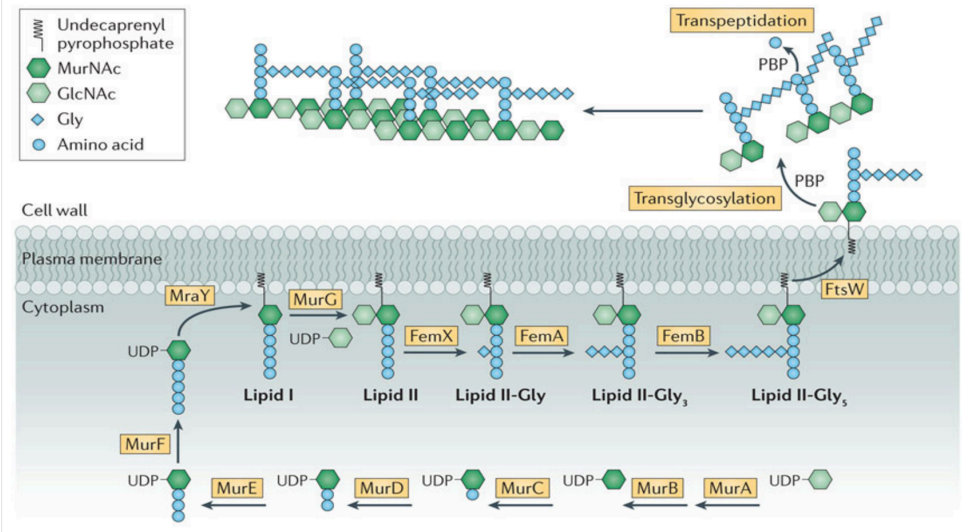

Figure 1. The peptidoglycan biosynthesis pathway. *The production of peptidoglycan monomers and subsequent transglycosylation and transpeptidation to form a cross-linked peptidoglycan polymer. Reproduced from Pinho et al (2013).*

The following components are needed as part of a 'molecular toolkit' to measure Lipid-II (Lys) Gly_5 formation via the FemXAB ligase assay, as illustrated in Fig. 1;

- FemX, A and B ligases
- Glycyl tRNA synthetase (GlyRS)
- *S. aureus* tRNA
- Amidated/Non-amidated Lipid-II (Lys)

FemX/A/B Expression & Purification

Method: FemX/A/B and GlyRS constructs were expressed in *Escherichia coli* BL21(DE3)*Rosetta, induced with 1 mM IPTG and harvested 4 hrs post induction, or after overnight expression. Proteins were purified via cobalt IMAC, reverse IMAC following His-tag cleavage (FemX) and gel filtration following pellet lysis and sonication.

Result: Approximate yields; 7.8 mg GlyRS, 4.5 mg FemX, 20 mg FemA and 1-2.6mg FemB.

Purification of *S. aureus* tRNA

Method: *S. aureus* ATCC 25923 (3L) was grown in LB at 37°C, 150 rpm overnight to OD 1 and pelleted. Pellets were lysed with lysostaphin and sonicated. tRNA was extracted using phenol, deacetylated and purified via anion exchange. Purified tRNA was precipitated, pelleted and resuspended in sterile dH_2O.

Result: Final yield 1 mg tRNA.

Comparison of L-II (Lys) synthesis

The *in vitro* production of Lipid-II (Lys) using *Micrococcus flavus* membranes is limited; yield produced using *M. flavus*, *S. epidermidis* and *S. aureus* membranes was compared.

Method: Cultures were grown to late exponential phase, pelleted and lysed using lysozyme (*M. flavus*), lysozyme and lysostaphin (*S. epidermidis*), or lysostaphin (*S. aureus*). Membranes were extracted following sequential centrifugation steps and used to produce L-II (Lys).

Result: L-II visualised via TLC (Fig. 2) and quantified via spectrophotometric assay (Data to be analysed). L-II production confirmed via mass spectrometry

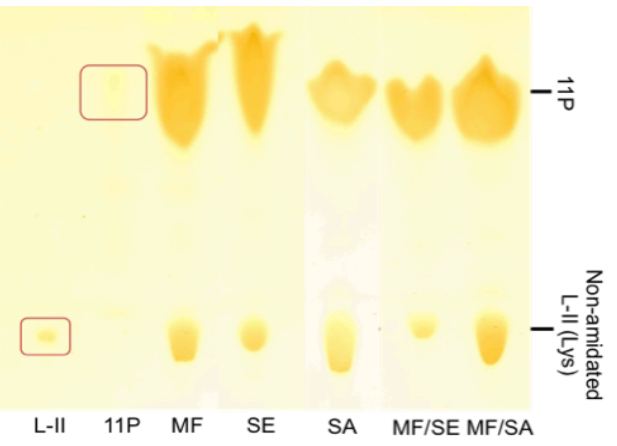

Figure 2. Comparison of Lipid-II production using bacterial membranes*. Lane 1; L-II control, lane 2; undecaprenyl phosphate (11P) control, L-II production using M. flavus (lane 3). S. epidermidis (lane 4), S. aureus (lane 5), M. flavus and S. epidermidis (lane 6) and M. flavus and S. aureus (lane 7) membranes.*

Future work

- Large scale L-II synthesis
- FemXAB Lipid-II assays to characterise lipid-II (Lys) penta-glycyl production (Radiochemically and non-radiochemically)
- Characterise FemXAB substrate affinity (NanoTemper or SPR)
- Cloning and expression of *S. aureus* MecA/PBP2a
- Biochemical analysis of PBP2a activity

References; Pinho *et al* (2013) Nature Reviews Microbiology. **11**. 601-614