	Experiment title:	Experiment number:
	Essential Bacillus subtilis gene products as targets for novel antimicrobial agents.	MX-508
Beamline: ID29	**Date of experiment:** from: 09 December 2005 to: 10 December 2005	**Date of report:** 25-6-07
Shifts: 2	**Local contact(s)** Dr Raimond Ravelli	*Received at ESRF:*

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Essential Bacterial gene products.

Bacterial pathogens are becoming of increasing importance in health care due to the spread of multi-antibiotic resistant strains. In order to develop new treatment regimes for infectious diseases it is crucial to identify novel therapeutic targets. Essential gene products are ideal, as they are required for cellular viability. In *Bacillus subtilis*, around 30 novel putative essential genes have been identified via functional genomics. Many of these are present in a wide range of bacterial pathogens. We are analysing these essential 30 genes from Bacillus and have initiated cloning, overexpression and purification of each, and have determined the structures of a number of these proteins, mostly by three-wavelength MAD data collected at the ESRF. This beamtime allocation was used to collect data from crystals of two such proteins: the *Bacillus subtilis* YkuR, and *Staphylococcus aureus* YmfM.

YkuR is a member of the metal containing M20 hydrolase superfamily. A knockout of this gene is bacteriocidal in *B. subtilis*, indicating that this enzyme is a good target for antimicrobial therapies. We have cloned, expressed and purified ykuR, however, diffraction quality crystals have proved very difficult to obtain. One seleno-methionine preparation of this protein, in the presence of Mn, yielded very thin plate crystals, in space group C222, with cell dimensions c=116.8, b=219.7, c= 158.0Å, with four copies of the polypeptide present in the asymmetric unit and a solvent content of 60%. Three wavelength MAD data were collected on ID29, however, the crystal only diffracted to a maximum resolution of 3.5 Å. Using this data the Se substructure could be determined using SHELXC/D/E and a preliminary electron density map produced. Four-fold molecular averaging yielded an interpretable map, into which the main chain and some side chains could be built. Rounds of rebuilding and refinement gave a final model with R and Rfree of 0.29 and 0.33, respectively.

The structure shows that the 374 amino acid YkuR molecule consists of two domains, the MH head domain (residues 1-173 and 288-374), and the M20 oligomerisation domain (residues 174-287).

Within the crystal four monomers come together to form a tetramer in 222 symmetry, assembled around the M20 domains, with each MH domain protruding form the centre to form a molecule with a stacked X appearance. The active site lies in the interface between the 2 domains, with the metal binding site in an equivalent position to that seen in the other MH clan members, yet utilising different amino acid side chains to coordinate the metal. Detailed analysis of the active site and is hampered by the low resolution of the data and a search for better diffracting crystals is underway.

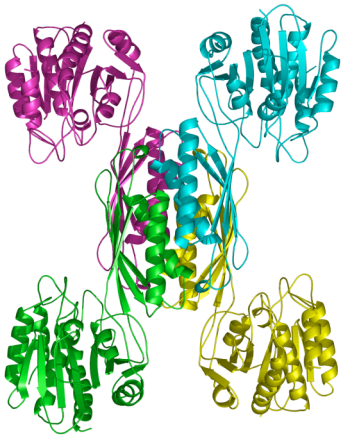

Figure 1, the molecular structure of *Bacillus subtilis* YkuR.

Data to 1Å resolution were also collected from a crystal of *S. aureus* YmfM, in space group $P4_32_12$, with cell dimensions a=b=45.5, c=72.9Å, with a monomer in the asymmetric unit. Sulphur SAD data had been previously collected at the SRS and although the 2 sulphur positions could be determined, the phase ambiguity could not be broken, due to the very low solvent content (22%). Using the ESRF 1Å data, and the 2 sulphur sites as seeds, the structure of this protein was determined by direct methods using SHELXD. The structure of *S. aureus* YmfM reveals that it comprises an antiparallel 5 helix bundle including a helix-turn-helix DNA binding motif. Structure comparisons show that the overall fold of YmfM reminiscent of the DNA binding domain of *Bacillus subtilis* SinR, a repressor involved in inhibiting sporulation. Biochemical studies have established that full length YmfM associates with the cell membrane presumably through its C-terminal hydrophobic segment and the soluble domain interacts with DNA. These data suggest that YmfM may be involved in regulating some aspect of DNA chemistry at the cell membrane possibly during cell division. A search for the function of YmfM including the identification of its DNA recognition sequence is underway in order to accelerate the understanding the role of this protein in virulence and pathogenicity in *S. aureus*.

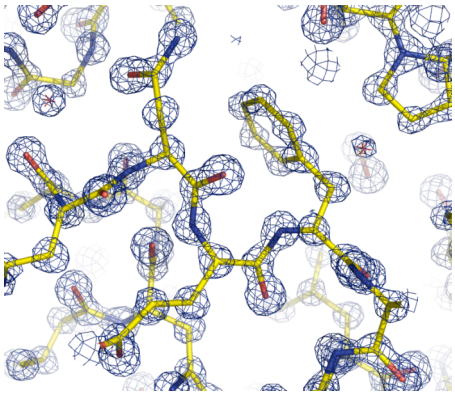

Figure 2. The electron density map of *S. aureus* YmfM.