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Supporting information for article:

Biochemical and biophysical characterization of the OXA-48-like carbapenemase OXA-436

Bjarte Aarmo Lund, Ane Molden Thomassen, Trine Josefine Warg Carlsen and Hanna-Kirsti Schrøder Leiros

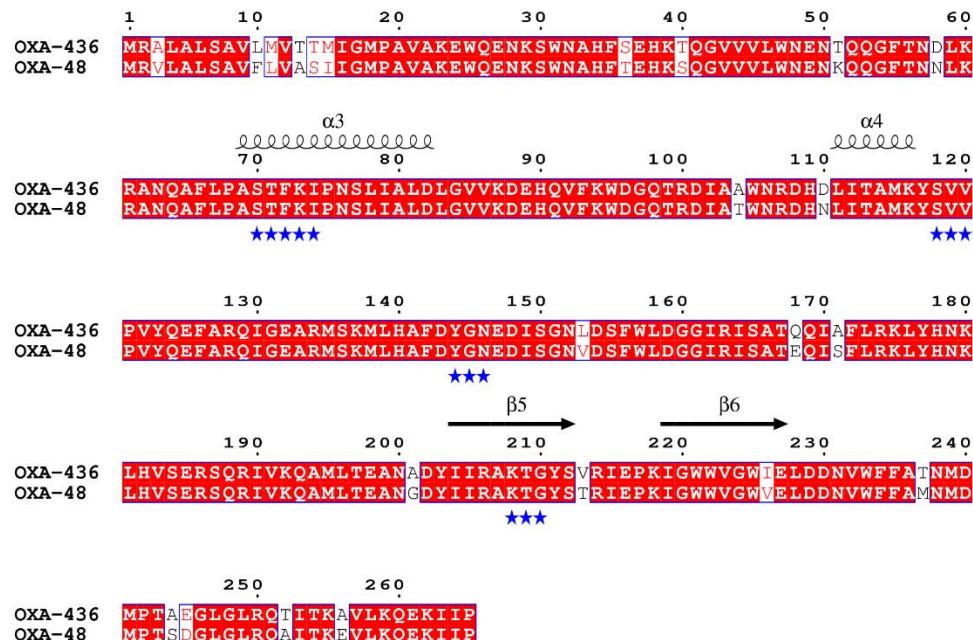


Figure S1 A pairwise sequence alignment of OXA-436 with OXA-48, with selected secondary structure elements numbered according to (Docquier *et al.*, 2009), shows the differences between the two sequences. Selected conserved structural typical to the Oxacillinases are marked with blue stars. The figure was prepared using ESPript (Robert & Gouet, 2014).

Table S1 Macromolecule production information

Source organism	<i>E. asburiae</i>
DNA source	Natural
Forward primer	ATAATTTGTTAACCTTAAGAAGGAGATACATATCGTGCCTAGCCTA
Reverse primer	CTTGTTAGCAGCCTCGAATCACTAAGGAATAATTTCCTGTTCA
Expression vector	pDEST17
Expression host	<i>E.coli</i> BL21 DE3 STAR pRARE
Complete amino acid sequence of the construct produced	<i>MSYYHHHHHLESTSLYGENLYFQG KEWQENKSWNAHFSEHKTQGVVVLWNENTQQGFTNDLK RANQAFPLASTFKIPNSLIALDLGVVKDEHQVFWDGQTRDIAAWNRDHDLITAMKYSVV PVYQEFAQRIGEARMSKMLHAFDYGNEDISGNLDSFWLDGGIRISATQQIAFLRKLYHNKLHVSERSQRIVKQAMLTEANADYIIRAKTGYSVRIEPKIGWWVGWIELDDNVWFFATNMD MPTAEGLGLRQTITKAVLKQEKEIIP</i>

The amino acid sequence in italics corresponds to the sequence inserted by the cloning procedure, and includes a TEV protease cleaving site which is used to enzymatically trim the sequence up to the final glycine before the start of the mature OXA-436 protein.

Table S2 :Crystallization parameters yielding OXA-436 crystals suitable for diffraction studies

Method	Hanging drop
Plate type	Hampton Research VDX 24 well
Temperature (K)	294
Protein concentration	13.8 mg/mL
Buffer composition of protein solution	50 mM HEPES pH 7.2
Composition of reservoir solution	0.1 M HEPES pH 7.75, 0.2 M sodium acetate, 26.5% PEG 3350
Volume and ratio of drop	1.5:1.5 µL
Volume of reservoir	1000 µL

Table S3 Primers for plasmid preparation and for exponential amplification

TEV protease site forward	ACCATCACCTCGAATCAACAAGTTGTACGGTGAGAATCTTATTTCAGGGTT
TEV protease site reverse	GGCTTGTTAGCAGCCTCGAATCAACCCTGAAAATAAGATTCTCACCG
EMP reverse	TTGTTGATTCGAGGTGATGGTGAT

References

- Docquier, J. D., Calderone, V., De Luca, F., Benvenuti, M., Giuliani, F., Bellucci, L., Tafi, A., Nordmann, P., Botta, M., Rossolini, G. M. & Mangani, S. (2009). *Chem Biol* **16**, 540-547.
Robert, X. & Gouet, P. (2014). *Nucleic Acids Research* **42**, W320-W324.