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Supplementary Information

Cloning, expression and purification of KSI-McjA fusion protein.

The *mcjA* gene (accession number Q9X2V7) was amplified by polymerase chain reaction using the plasmid pTUC202 as a template, and the resulting 195 bp fragment was subsequently inserted into the pET-31b expression vector (Novagen) using *AlwNI* restriction sites. The resulting construct, pET-31b/*mcjA*, contained the *mcjA* gene downstream of a keto-steroid isomerase gene (*ksi*), and was used to express the KSI-McjA fusion protein in *E. coli* as insoluble inclusion bodies (supplementary Figure 1). The fidelity of pET-31b/*mcjA* was verified by DNA sequencing, before transformation of *E. coli* BL21(DE3) Rosetta 2 (Novagen) cells. These cells were grown in 2YT growth media supplemented with ampicillin (100 μg/ml) and chloramphenicol (30 μg/ml) in shake flasks at 37 °C and 250rpm to $OD_{600} = 0.3$ before induction with isopropyl-1-thio-β-D-galactopyranoside (IPTG, 1.0 mM final concentration). After a further 6 hours, the cells were collected by centrifugation and the cell pellet was stored at 4 °C.

The cell pellet was resuspended in 5ml/ g wet weight resuspension buffer (50 mM Tris, 25% (w/v) sucrose, 1 mM EDTA, 10 mM DTT, pH 8.0) before addition of 50 mg lysozyme and 5 units DNAse I. Lysis buffer (50 mM Tris, 1% (w/v) Triton-X100, 100 mM NaCl, 10 mM DTT, pH 8.0) was then added (5ml/ g wet weight) before the cell suspension was incubated at room temperature for 45 minutes. 700 μl EDTA (500 mM) was then added and the suspension was snap frozen in liquid nitrogen and then thawed at 37 °C. Subsequently, 500 μl of $MgCl_2$ (500 mM) was added and the suspension was incubated at room temperature for 30 minutes before the crude inclusion bodies were collected by centrifugation at 12,000*g* for 20 minutes at 4 °C. The inclusion bodies were then washed three times with wash buffer (50 mM Tris, 1% (w/v) Triton-X100, 100 mM NaCl, 1 mM DDT, 1 mM EDTA pH 8.0), until the 20kDa KSI-McjA fusion-protein was judged >90% pure by SDS-PAGE. Finally, the fusion protein was dissolved in a minimum quantity of solubilisation buffer (50 mM Tris, 6 M Guanidine HCl, pH 8.0) and any insoluble debris was removed by centrifugation at 18,000*g* for 20 minutes at 4 °C. The fusion protein was then precipitated by addition of chilled water to dilute the guanidine to a final concentration of 1 M, before the purified fusion protein was collected by centrifugation at 12,000*g* for 20 minutes at 4 °C.

Chemical cleavage of the KSI-McjA fusion protein and purification of McjA.

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The fusion protein was dissolved in a minimum quantity of 70 % (v/v) formic acid and was treated with 0.5 M cyanogen bromide. The reaction was performed under nitrogen at 22 °C and was allowed to proceed for 18 hours in the dark. After completion, the mixture was then evaporated by 2/3 volume under vacuum and freeze dried. The cleaved McjA peptide was extracted from the mixture with 25 mM phosphate buffer, pH 7.5. Finally, the McjA was purified by cation exchange chromatography on a Mono-S column using a linear 0-1 M NaCl gradient (attached to an AKTA-FPLC system (GE healthcare). SDS-PAGE analysis was used to verify that the McjA protein (~6kDa) was >98% pure and LC-MS analysis revealed that the protein has a mass of 6068.0 Da (which is consistent with the theoretical value of 6068.1 Da).

A

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  1 MHTPEHITAV VQRFVAALNA GDLDGIVALF ADDATVEDPV GSEPRSGTAA
 51 REFYANSLK LPLAVELTQE VRAVANEAAF AFTVSFEYQG RKTVVAPIDH
101 FRFNGAGKVV SIRALFGEKN IHACQMLMIK HFHFNKLSSG KKNNVPSPAK
151 GVIQIKKSAS QLTKGGAGHV PEYFVGIGTP ISFYG
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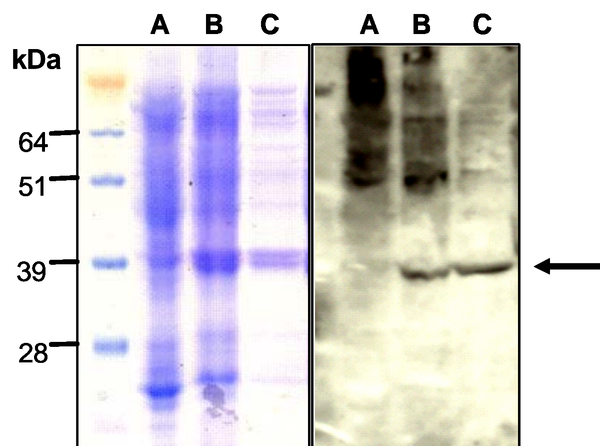
B

NH_2-IKHFHFNKLSSGKKNNVPSPAKGVIQIKKSASQLTKGGAGHVPEYFVGIGTPISFYG-COOH

Supplementary Figure 1 (**A**) Sequence of the insoluble KSI-McjA fusion product. The sequence highlighted in red is the McjA peptide. (**B**) Sequence of the McjA precursor peptide produced by CNBr cleavage of the KSI-McjA fusion used in McjA maturation experiments. The sequence highlighted in blue is the 21 residue MccJ25 mature microcin.

Western Blot analysis demonstrating McjC is membrane associated.



Supplementary Figure 2. SDS-PAGE (left) and Western Blot (right) of *pTUC203 C6H* expressing cells. **A**, soluble protein; **B**, insoluble protein; **C**, membrane protein extracted with 1% DDM. The band indicated with an arrow which migrates with an apparent molecular weight of ~39 kDa is histidine tagged McjC.

Purification of MccJ25.

MccJ25 was typically purified from 3 l cultures of *E. coli* Novablue transformed with *pTUC202*, as previously described.[11] The cells were grown in M9 minimal medium until stationary phase and, after removing the bacteria by centrifugation at 12000*g* for 30 min, the supernatant was applied to a preparative C8 cartridge (BondElut, Varian). The column was subsequently washed with methanol:water, 20:80 (v/v) and then 50:50 (v/v), before the microcin was eluted with 4 x 5 ml of methanol:water, 80:20 (v/v). Crude MccJ25 was further purified using reverse phase-high performance liquid chromatography (RP-HPLC) on a Jupiter Proteo Column 250 x 10.00mm C12 (Phenomenex) and analysed by ESI-MS (see Fig. 3C).