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The cloning, crystallization and preliminary X-ray analysis of XC2113, a YaeQ protein from *Xanthomonas campestris*

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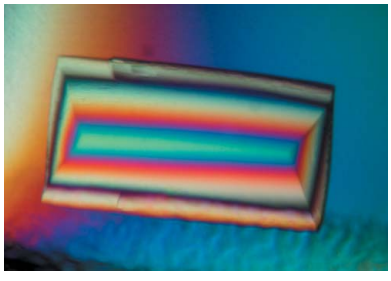
Xanthomonas campestris is a Gram-negative bacterium that is phytopathogenic to cruciferous plants and causes worldwide agricultural loss. It is therefore important to identify potential pathogenic factors involved in this plant disease. Here, the cloning, expression, crystallization and preliminary X-ray analysis of XC2113, a YaeQ protein possibly involved in the production of virulence factors in *Xanthomonas campestris* pathovar *campestris*, are reported. The XC2113 crystals diffracted well to a resolution of at least 1.28 Å. They are orthorhombic and belong to space group $P2_12_12_1$, with unit-cell parameters $a = 32.86$, $b = 62.69$, $c = 79.96$ Å.

1. Introduction

XC2113 (gi|21113430) from the plant pathogen *Xanthomonas campestris* pathovar *campestris* strain 17 (Xcc) is a YaeQ protein identified from a sequence match using the Pfam database (Bateman *et al.*, 2000). It belongs to a family of bacterial proteins that are highly conserved in Gram-negative pathogens and was initially suggested to be involved in the transcription regulation of genes encoding virulence factors such as haemolysin (Wong *et al.*, 1998). However, as further research indicated that YaeQ does not directly affect virulence-factor transcription (Vicari & Artsimovitch, 2004), it was suggested that YaeQ may affect the synthesis or secretion of some other haemolytic factors at the post-transcription level or that it could possess a cryptic haemolytic activity on its own. These hypotheses are corroborated by the presence of a C-terminal element in *Escherichia coli* YaeQ that is weakly homologous to a thermostable haemolysin from *Vibrio* sp. (IPB005015D; Vicari & Artsimovitch, 2004). However, as YaeQ does not possess other significant signatures that would suggest a model for its function, the precise function of this protein still remains unknown. Hence, its three-dimensional structure is crucial in order to obtain further functional insights. To this end, we have cloned, expressed, crystallized and performed preliminary X-ray analysis of XC2113, a YaeQ protein from the Xcc genome. The XC2113 crystals were found to diffract well to a resolution of at least 1.28 Å. The recombinant XC2113 comprises 185 amino-acid residues (including an extra SNA tripeptide at the N-terminal end) with a molecular weight of 20 892.45 Da. Recently, a YaeQ protein from another similar *Xanthomonas* species (*X. axonompdis* pv. *citri*) has also been crystallized and diffracted to a resolution of 1.9 Å (Guzzo *et al.*, 2005).



2. Materials and Methods

2.1. Cloning, expression, and purification

The XC2113 gene fragment was PCR-amplified directly from a local Xcc genome with a forward 5′-TACTTCCAATCCAATGCTATGGCAGACAACGTTGCGGGCA primer and a reverse 5′-TTATCCACTTCCAATGTCAGTTCTGCACACCGCCGCCGTT primer. A ligation-independent cloning (LIC) approach (Aslanidis & de Jong, 1990) was carried out to obtain the desired construct according to a previously published protocol (Wu *et al.*, 2005). The final construct codes for an N-terminal His_6 tag, a 17-amino-acid linker and the XC2113 target protein (182 amino acids) under the

control of a T7 promoter. Transformed *E. coli* BL21 (DE3) host cells were grown in LB medium at 310 K until an OD_{600} of 0.8 was attained. Overexpression of the His_6-tagged target protein was induced by the addition of 0.5 m*M* IPTG at 310 K for 4 h. The cells were harvested, resuspended in equilibration buffer (20 m*M* Tris pH 8.0, 70 m*M* NaCl) and lysed using a microfluidizer (Microfluidics). Most tagged target protein remains in the soluble fraction (Fig. 1). After centrifugation, the target protein was purified by immobilized metal-affinity chromatography (IMAC) on a nickel column (Sigma), which was eluted with 20 m*M* Tris pH 8.0, 70 m*M* NaCl and a gradient of 100–300 m*M* imidazole. The fractions containing XC2113 were monitored by SDS–PAGE, recombined and dialyzed repeatedly against 50 m*M* Na_2HPO_4 pH 8.0, 10% glycerol and 500 m*M* NaCl. After buffer exchange, the His_6 tag and linker were cleaved from XC2113 using TEV (tobacco etch virus) protease at 295 K for 12 h. The purified protein was dialyzed against 20 m*M* Tris pH 8.0 and 70 m*M* NaCl several times. For crystallization, XC2113 was further purified on an anion-exchange column (AKTA, Pharmacia Inc.). The fractions eluted with 20 m*M* Tris pH 8.0, 500 m*M* NaCl were combined and dialyzed against 20 m*M* Tris pH 8.0 and 70 m*M* NaCl. The final target protein (182 amino acids) has a greater than 99% purity (Fig. 1) and contains only an extra tripeptide (SNA) at the N-terminal end. The overexpression and purification of XC2113 were monitored by SDS–PAGE as shown in Fig. 1.

2.2. Crystallization

For crystallization, the protein was concentrated to 35 mg ml^{-1} in 20 m*M* Tris pH 8.0 and 70 m*M* NaCl using an Amicon Ultra-10 (Millipore). Screening for crystallization conditions was performed using the sitting-drop vapour-diffusion method in 96-well plates (Hampton Research) at 293 K by mixing 0.5 µl protein solution with 0.5 µl reagent solution. Initial screens included the Hampton sparse-matrix Crystal Screens 1 and 2, a systematic PEG–pH screen and the PEG/Ion Screen and were performed using a Gilson C240 crystallization workstation. Needle-like crystals appeared in 3 d from a reservoir solution comprising 0.1 *M* Tris buffer pH 8.5, 0.2 *M* $MgCl_2$ and 30%(*w*/*v*) PEG 4K. Trigonal pillar-shaped crystals suitable for diffraction experiments were grown by mixing 0.5 µl protein solution with 0.5 µl reagent solution at room temperature and reached maximum dimensions of 0.4 × 0.2 × 0.2 mm after two weeks (Fig. 2).

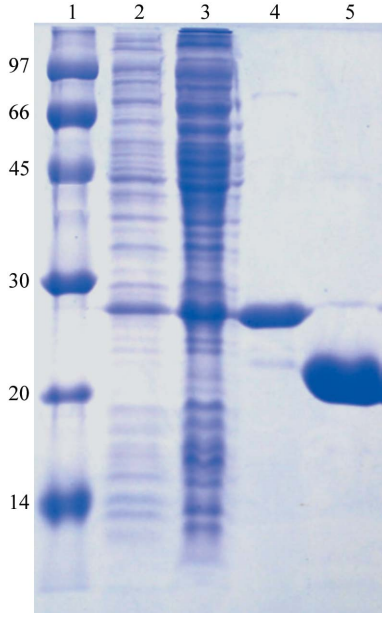


Figure 1
SDS–PAGE monitoring of the overexpression and purification of XC2113. Lane 1, molecular-weight markers in kDa; lane 2, whole cell lysate before IPTG induction; lane 3, whole cell lysate after IPTG induction; lane 4, purified XC2113 before TEV cleavage; lane 5, purified XC2113 after TEV cleavage. A single band of correct MW of greater than 99% purity was observed.

Table 1
Data-collection statistics for XC2113.

Values in parentheses are for the highest resolution shell.

Space group	$P2_12_12_1$
Unit-cell parameters (Å)	a = 32.86, b = 62.69, c = 79.96
Temperature (K)	100
Wavelength (Å)	0.97622
Resolution range (Å)	30.0–1.28 (1.33–1.28)
Mosaicity (°)	0.4
Unique reflections	248191 (5891)
Redundancy	5.8 (5.5)
Completeness (%)	98.9 (99)
R_{sym}† (%)	3.8 (21)
Mean $I/\sigma(I)$	15.7 (14.5)
Solvent content (%)	39.8

† $R_{sym} = \sum_{\mathbf{h}} \sum_l |I_{\mathbf{h}l} - \langle I_{\mathbf{h}} \rangle| / \sum_{\mathbf{h}} \sum_l \langle I_{\mathbf{h}} \rangle$, where I_l is the lth observation of reflection **h** and $\langle I_{\mathbf{h}} \rangle$ is the weighted average intensity for all observations l of reflection **h**.

2.3. Data collection

Crystals were directly flash-cooled in a stream of cold nitrogen at 100 K in the mother liquor, which served as a cryoprotectant. X-ray diffraction data were collected on the 13B1 beamline of the National Synchrotron Radiation Research Center (NSRRC) facility, Taiwan using an ADSC Quantum 210 CCD detector. A native data set reaching 1.28 Å resolution was obtained. The data were indexed and integrated using the *HKL*-2000 processing software (Otwinowski & Minor, 1997), giving a data set that was 98.9% complete with an overall R_{sym} of 3.8% on intensities. The crystals belong to the orthorhombic space group $P2_12_12_1$. The data-collection statistics are summarized in Table 1; an X-ray diffraction image collected at the NSRRC facility is shown in Fig. 3.

3. Results and discussion

The XC2113 gene consisted of 549 bp coding for 182 amino-acid residues. The isoelectric point was calculated to be 5.16. The purified

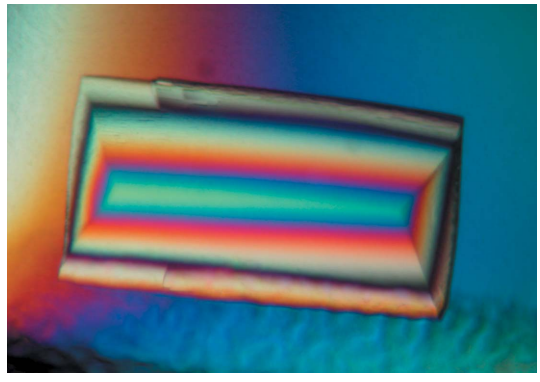


Figure 2
Trigonal pillar-shaped crystals of XC2113 from Xcc grown by the hanging-drop vapour-diffusion method. The crystallization condition used was 0.1 *M* Tris buffer pH 8.5, 0.2 *M* $MgCl_2$ and 30%(*w*/*v*) PEG 4K. The average dimensions of these crystals were all around 0.4 × 0.2 × 0.2 mm.

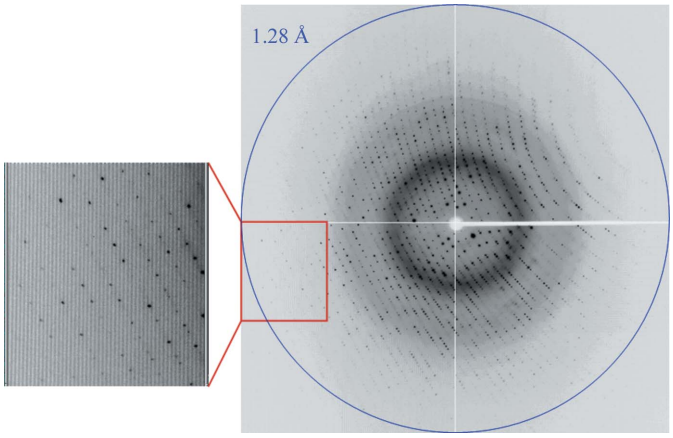


Figure 3
The diffraction pattern of XC2113 collected on the NSRRC 13C1 beamline from a crystal flash-cooled in reservoir cryoprotectant. The exposure time was 5 s, with an oscillation range of 1° and a crystal-to-detector distance of 105 mm.

XC2113 contains an extra SNA tripeptide at the N-terminal end and shows a single band of approximate 20 kDa on SDS–PAGE (Fig. 1), which is in good agreement with the calculated MW of 20 892.45 Da.

The high-resolution diffraction obtained from the native crystals establishes their suitability for further X-ray structural analysis (Fig. 3). The crystal belongs to space group $P2_12_12_1$ as indicated by the systematic absences of reflections along the a^*, b^* and c^* directions, with unit-cell parameters $a = 32.86$, $b = 62.69$, $c = 79.96$ Å. The Matthews coefficient (V_M = 2.1 Å^3 Da^{-1}; solvent content 39.8%) indicates that there is only one protein molecule per asymmetric unit.

While this work was in progress, the crystallization and preliminary X-ray data of a similar YaeQ protein of identical sequence from another *Xanthomonas* species (*X. axonompdis* pv. *citri*) were published (Guzzo *et al.*, 2005). Two different reservoir solutions were used in the studies of Guzzo and coworkers: [30–32%(*w*/*v*) PEG 4K, 0.2 *M* ammonium acetate and 0.1 *M* Tris–HCl pH 8.5–9.0 or 30%(*w*/*v*) PEG 8K, 0.1 *M* sodium cacodylate pH 6.7 and 0.2 *M* sodium acetate] and similar hexagonal plate-shaped crystals of dimensions 0.4 × 0.4 × 0.03 mm were obtained. The crystals were monoclinic and diffracted to a resolution of 1.9 Å. In our case, we have screened out a different reservoir-solution condition [0.1 *M* Tris buffer pH 8.5, 0.2 *M* $MgCl_2$, 30%(*w*/*v*) PEG 4K] using a Gilson C240 crystallization workstation and obtained trigonal pillar-shaped crystals (Fig. 2) belonging to the orthorhombic system with dimensions of up to 0.4 × 0.2 × 0.2 mm. Very good X-ray diffraction data up to 1.28 Å could be obtained from these crystals. Thus, although the protein sequences are identical, different crystal forms with different dimensions were obtained for the YaeQ protein under different crystallization conditions. It remains to be seen if such crystals will result in similar or different conformations of the YaeQ protein. Since no tertiary structure for the YaeQ protein has been published to date, we plan to solve its phases and structure by the single-wavelength anomalous diffraction (SAD; Wang, 1985; Dauter, 2002) method or the multiwavelength anomalous diffraction (MAD; Hendrickson & Ogata, 1997; Terwilliger & Berendzen, 1999) method using selenomethionine-substituted protein. This work is now in progress.

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