Structure of a thermostable serralysin from *Serratia* sp. FS14 at 1.1 Å resolution

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Serralysin is a well studied metalloprotease, and typical serralysins are not thermostable. The serralysin isolated from *Serratia* sp. FS14 was found to be thermostable, and in order to reveal the mechanism responsible for its thermostability, the crystal structure of serralysin from *Serratia* sp. FS14 was solved to a crystallographic *R* factor of 0.1619 at 1.10 Å resolution. Similar to its homologues, it mainly consists of two domains: an N-terminal catalytic domain and a 'parallel β -roll' C-terminal domain. Comparative studies show that the shape of the catalytic active-site cavity is more open owing to the 189–198 loop, with a short 3_{10} -helix protruding further from the molecular surface, and that the β -sheets comprising the 'parallel β -roll' are longer than those in its homologues. The formation of hydrogen bonds from one of the nonconserved residues (Asn200) to Lys27 may contribute to the thermostability.

1. Introduction

The Gram-negative bacteria *Serratia* spp. are facultative anaerobic organisms that ubiquitously inhabit soil, water, animals, plants and humans. *Serratia* species can secrete a variety of exoenzymes including proteases, nucleases, phospholipases and chitinases (Monreal & Reese, 1969; Braun & Schmitz, 1980; Givskov *et al.*, 1988; Chen *et al.*, 1992; Petersen & Tisa, 2014). In particular, serralysin, a zinc-dependent metalloprotease, has been well studied (Miyata *et al.*, 1970; Braunagel & Benedik, 1990; Baumann, 1994; Baumann *et al.*, 1995; Aiyappa & Harris, 1976; Hamada *et al.*, 1996).

Serralysin has been found to not only play important roles in bacterial nutrition but also in virulence as a cytotoxic factor capable of infecting larvae, mammalian cells and the human immune system (Marty *et al.*, 2002; Kida *et al.*, 2007; Petersen & Tisa, 2014; Shanks *et al.*, 2015; Pineda-Castellanos *et al.*, 2015).

Serralysin homologues have also been found in many other enteric bacteria including *Erwinia chrysanthemi* (Delepelaire & Wandersman, 1989), *Pseudomonas aeruginosa* (Mori-hara & Okuda, 1992) and *Photobacterium luminescens* (Daborn *et al.*, 2001). These serralysin-family proteases share approximately 50% sequence similarity with each other. Serralysins are usually not thermostable and their proteolytic activities decline rapidly above 315 K, with no detectable activity above 328 K (Miyata *et al.*, 1971; Liang *et al.*, 2010; Sarker & Cornelis, 1997), with the exception of the serralysin isolated from *Serratia* sp. RSPB11, which retains 45% of its protease activity after pretreatment at 343 K for 15 min (Bhargavi & Prakasham, 2013).

The first crystal structure of serralysin to be reported was that from *S. marcescens* (Baumann, 1994). To date, 16 crystal structures of serralysin-family proteases have been deposited

in the Protein Data Bank (Berman *et al.*, 2000) with resolutions from 1.64 to 2.8 Å, including native serralysins or serralysin homologues and serralysins or serralysin homologues complexed with inhibitors or ligands, but only three of them are from *Serratia* species [PDB entries 1sat (Baumann, 1994), 1smp (Baumann *et al.*, 1995) and 1srp (Hamada *et al.*, 1996)], with resolutions of 1.8, 2.3 and 2.0 Å, respectively. Structurally, the catalytic domain of serralysin comprises a large N-terminal lobe mainly consisting of a five-stranded mixed β -sheet and three α -helices, and a small C-terminal lobe mainly assembled from an α -helix and an irregular structure. The Zn^{2+} -binding pocket is located in a groove between the lobes. Inhibitor binding leads to movement of both lobes, and the loop region between Asp189 and Thr198 with high temperature factors seems to be relatively flexible, which induces opening and closure of the catalytic cleft (Hamada *et al.*, 1996).

Previously, we isolated the bacterial strain *Serratia* sp. FS14 from the stem of diseased *Atractylodes macrocephala* Koidz and found that it could secrete a thermostable protease (Zheng *et al.*, 2011). A 50 kDa protease from the supernatant culture of *Serratia* sp. FS14 was identified as a serralysin and a thermostability assay showed that it retained 40% of its protease activity after pretreatment at 363 K for 10 min.

To dissect the thermostable enzymatic properties of serralysin from *Serratia* sp. FS14 at the structural level, we determined the crystal structure of serralysin from *Serratia* sp. FS14 at the atomic resolution of 1.1 Å.

2. Materials and methods

2.1. Purification and protease-activity assay

Unless otherwise noted, all purification steps were carried out at 277 K.

Serratia sp. FS14 (Zheng *et al.*, 2011) cells were grown in Luria–Bertani (LB) broth (1.0% tryptone, 0.5% yeast extract, 1.0% NaCl pH 7.0) on a rotary shaker at 180 rev min⁻¹ at 301 K for 12 h and transferred to 1 l LB medium at 301 K for a further 24 h. The culture was centrifuged at 16 000g for 30 min; the supernatant was passed through 0.45 and 0.22 µm filters and was then precipitated by ammonium sulfate at 80% saturation. The precipitate was collected after centrifugation at 24 000g for 30 min and dissolved in 20 ml 100 mM sodium phosphate buffer pH 7.0. After centrifugation at 24 000g for 30 min, the supernatant was collected, diluted with an equal volume of 3 M ammonium sulfate and loaded onto a CM Sephadex C-50 hydrophobic interaction chromatography column pre-equilibrated with buffer consisting of 1.5 M ammonium sulfate, 50 mM sodium phosphate pH 7.0. The column was eluted with a linear gradient of 1.5–0 M ammonium sulfate in 50 mM sodium phosphate buffer pH 7.0. The fractions with thermostable protease activity were pooled and dialyzed against 200 volumes of a solution consisting of 20 mM Tris–HCl buffer pH 8.0 for 12 h and then applied onto a DEAE Sephadex A-50 anion-exchange chromatography column pre-equilibrated with 20 mM Tris–HCl buffer pH 8.0 and eluted with a linear gradient of 0–1 M NaCl in 20 mM

Table 1

Data collection and processing.

Values in parentheses are for the outer shell.

Diffraction source	BL17U, SSRF
Wavelength (Å)	0.9792
Temperature (K)	100
Detector	ADSC Q315r
Crystal-to-detector distance (mm)	120
Rotation range per image (°)	1.0
Total rotation range (°)	360
Exposure time per image (s)	1.0
Space group	$P2_12_12_1$
a, b, c (Å)	42.3, 105.4, 151.1
Mosaicity (°)	0.18
Resolution range (Å)	20–1.1 (1.2–1.1)
Total No. of reflections	1915565 (423363)
No. of unique reflections	273151 (62101)
Completeness (%)	99.5 (99.5)
$R_{\text{merge}}^{\dagger}$ (%)	9.1 (93.2)
$R_{\text{meas}}^{\ddagger}$ (%)	9.9 (100.9)
$\langle I/\sigma(I) \rangle$	10.96 (2.03)
Overall B factor from Wilson plot (Å ²)	13.6

$\dagger R_{\text{merge}} = \sum_{hkl} \sum_i |I_i(hkl) - \langle I(hkl) \rangle| / \sum_{hkl} \sum_i I_i(hkl)$, where $I_i(hkl)$ and $\langle I(hkl) \rangle$ are the observed intensity of the i th measurement and the mean intensity of the reflection with indices hkl , respectively. $\ddagger R_{\text{meas}} = \sum_{hkl} \{N(hkl)/[N(hkl) - 1]\}^{1/2} / \sum_i |I_i(hkl) - \langle I(hkl) \rangle| / \sum_{hkl} \sum_i I_i(hkl)$.

Tris–HCl buffer pH 8.0. The fractions with thermostable protease activity were then concentrated and applied onto a Superdex 200 column (30 × 1.0 cm) which had been pre-equilibrated with 20 mM Tris–HCl pH 8.0, 0.3 M NaCl. The protease was finally eluted with the same buffer. The purified protease was inspected on 10% SDS–PAGE and stored at 277 K for further crystallization and protease-activity assays. Protease activity was determined using a reaction mixture consisting of 50 µg casein and 5 µg purified protein in 50 mM Tris–HCl pH 8.0, 20 mM NaCl buffer at 310 K for 10 min using the method described by Saleemuddin *et al.* (1980).

2.2. Crystallization and data collection

After purification, the protein samples were concentrated to 20 mg ml⁻¹. Crystals suitable for data processing were grown in 24-well plates by the hanging-drop vapour-diffusion method by mixing 1 µl protein solution with 1 µl reservoir solution and equilibrating against reservoir solution consisting of 0.1 M ammonium sulfate, 0.1 M sodium citrate pH 6.3, 30% (w/v) polyethylene glycol (PEG) 8000. The best crystals were obtained at 289 K in 4 d.

Crystals were looped out from the crystallization drop and transferred into fresh mother liquor with 10% (w/v) glycerol as a cryoprotectant for several seconds. The crystal was then flash-cooled in liquid nitrogen. Diffraction data were collected on beamline BL17U at Shanghai Synchrotron Radiation Facility (SSRF), Shanghai, People's Republic of China. The raw data were processed using the XDS package (Kabsch, 2010). Data-collection and processing statistics are summarized in Table 1.

2.3. Structure determination and refinement

The molecular-replacement method was used to solve the structure of serralysin from *Serratia* sp. FS14 using the

Table 2

Structure solution and refinement.

Values in parentheses are for the outer shell.

PDB code	5d7w
Resolution range (Å)	20–1.1
Total No. of atoms	3474
No. of reflections used	259368
R_{work} (%)	16.19
R_{free} (%)	17.553
R.m.s.d., bond lengths (Å)	0.0064
R.m.s.d., bond angles (°)	1.085
Average B factors (Å ²)	
Protein	12.74
Water	27.66
Ramachandran plot (%)	
Favoured	96.25
Allowed	3.75
Outliers	0

structure of the 50 kDa metalloprotease SMP from *S. marcescens* (PDB entry 1sat; Baumann, 1994) as a template. Molecular replacement was performed using *Phaser* (McCoy *et al.*, 2007), the model was subsequently manually adjusted and checked with *Coot* (Emsley *et al.*, 2010) and refinement was carried out using *REFMAC5* (Murshudov *et al.*, 2011) and *PHENIX* with isotropic B factors (Adams *et al.*, 2010). Structure-solution and refinement statistics are summarized in Table 2.

3. Results and discussion

3.1. Purification of the thermostable serralysin

By monitoring the protease activity during purification, the thermostable serralysin was purified from the culture supernatant of *Serratia* sp. FS14 in several steps. A protease-activity assay showed that this serralysin has about 40% protease activity remaining after pretreatment at 363 K for 10 min. The serralysin is secreted in a large amount, with a yield of 10 mg purified protein per litre of culture supernatant.

3.2. Overall structure

The crystal structure of serralysin from *Serratia* sp. FS14 was solved and refined to a crystallographic R factor of 0.1619 at 1.1 Å resolution (Table 2). Serralysin from *Serratia* sp. FS14 is an elongated molecule with approximate dimensions of $90 \times 35 \times 40$ Å. It is comprised of an extended N-terminal tail (residues 3–20) contacting the C-terminus and two main domains: the N-terminal proteolytic domain (residues 21–251) and the ‘parallel β -roll’ C-terminal domain (residues 252–471) (Fig. 1).

Furthermore, the N-terminal domain is divided into an upper N-lobe consisting of a five-stranded β -sheet and three α -helices and a lower C-lobe consisting mostly of irregular structure with an α -helix and three short 3_{10} -helices. The lobes form a large flap-shaped active-site cleft hosting a zinc ion at the bottom. The β -sandwich C-terminal domain consists of 16 β -strands, three short 3_{10} -helices and irregular structure, characterized by an elongated parallel β -roll binding seven calcium ions.

3.3. Structural comparison with serralysin homologues

The sequence of serralysin from *Serratia* sp. FS14 shares 98% identity with two homologues for which structures have been determined (Fig. 2). The overall structure of serralysin reported here (PDB entry 5d7w) shares a similar fold with its homologues, such as SMP (serralysin) from *S. marcescens* (PDB entry 1sat; Baumann, 1994; r.m.s.d. of 0.18 Å for 408 C α atoms) and *Serratia* protease (serralysin) from *Serratia* sp. E-15 (PDB entry 1srp; Hamada *et al.*, 1996; r.m.s.d. of 0.20 Å for 407 C α atoms).

Sequence alignment showed that only nine residues differ among the three *Serratia* serralysins (Fig. 2). Nevertheless, several structure differences can obviously be observed. One obvious difference is residue Asn200 (Arg in *Serratia* protease and Ala in SMP), which forms hydrogen bonds to Lys27; at the same time Lys27 also forms hydrogen bonds to the carbonyl groups of Asn200 and Val202 (labelled 1 in Fig. 3a). These interactions are not present in the other *Serratia* serralysins. The interactions between Lys27 and Asn200 and Val202 may stabilize the protein. This phenomenon has also been observed recently by Zhang and coworkers, who introduced interactions between the N-terminal helix and the RTX domain, leading to an increase in the thermal and chemical stability, of serralysin from *S. marcescens* (Zhang *et al.*, 2015).

Figure 1

Cartoon view of the overall structure of serralysin, coloured according to secondary-structure element. Calcium ions (Ca1–Ca7) are shown as green spheres. The zinc ion is shown as a grey sphere with its ligands in yellow.

Additionally, the 189–198 loop with a short 3_{10} -helix in serralysin from *Serratia* sp. FS14 protrudes further away from the molecular surface than the 189–198 loop in SMP or

Serratia protease, restricting the entrance to the active-site cavity. This loop is ordered in the *Serratia* sp. FS14 structure and has relatively high *B* factors ($28.95 \text{ \AA}^2$) compared with the

	3					
1	AAATGYDAVDDLLHYHERNGI	QINGKDSFSNEQAGLFITRENQ	TWNGYKVF	QGPVKLTF	60	Serralyisin (5D7W)
1	AAATGYDAVDDLLHYHERNGI	QINGKDSFSNEQAGLFITRENQ	TWNGYKVF	QGPVKLTF	60	SMP (1SAT)
1	AAATGYDAVDDLLHYHERNGI	QINGKDSFSNEQAGLFITRENQ	TWNGYKVF	QGPVKLTF	60	Serratia protease (1SRP)
61	SFPDYKFSSTNVAGDTGLSKFSAEQ	QQQAKLSLQSWADV	ANITFTEVAAGQKANITFGNY	120	Serralyisin (5D7W)	
61	SFPDYKFSSTNVAGDTGLSKFSAEQ	QQQAKLSLQSWADV	ANITFTEVAAGQKANITFGNY	120	SMP (1SAT)	
61	SFPDYKFSSTNVAGDTGLSKFSAEQ	QQQAKLSLQSWADV	ANITFTEVAAGQKANITFGNY	120	Serratia protease (1SRP)	
121	SQDRPGHYDYG	TQAYAF	LPTNIWQGQDLGGQ	TWYNVNQSNVKHPATEDYGRQTF	THEIGH	180 Serralyisin (5D7W)
121	SQDRPGHYDYG	TQAYAF	LPTNIWQGQDLGGQ	TWYNVNQSNVKHPATEDYGRQTF	THEIGH	180 SMP (1SAT)
121	SQDRPGHYDYG	TQAYAF	LPTNIWQGQDLGGQ	TWYNVNQSNVKHPATEDYGRQTF	THEIGH	180 Serratia protease (1SRP)
	196	200				
181	ALGLSHPGDYNAGEG	PTYNDV	TYAEDTRQFSLMSY	WSETNTGGDNGGHY	AAAPLLDDIA	240 Serralyisin (5D7W)
181	ALGLSHPGDYNAGEG	PTYNDV	TYAEDTRQFSLMSY	WSETNTGGDNGGHY	AAAPLLDDIA	240 SMP (1SAT)
181	ALGLSHPGDYNAGEG	PTYNDV	TYAEDTRQFSLMSY	WSETNTGGDNGGHY	AAAPLLDDIA	240 Serratia protease (1SRP)
241	AIQHLYGANLSTR	TGDTVYGFNSNTGRD	FLSTTSNSQKVI	FAAWDAGGNDT	FDFSGYTAN	300 Serralyisin (5D7W)
241	AIQHLYGANLSTR	TGDTVYGFNSNTGRD	FLSTTSNSQKVI	FAAWDAGGNDT	FDFSGYTAN	300 SMP (1SAT)
241	AIQHLYGANLSTR	TGDTVYGFNSNTGRD	FLSTTSNSQKVI	FAAWDAGGNDT	FDFSGYTAN	300 Serratia protease (1SRP)
301	QRINLNEKSFSDV	GGLKGNVSI	AAGVTIENAI	GGSGNDVIVGNAANNVLKGGAGNDV	LFG	360 Serralyisin (5D7W)
301	QRINLNEKSFSDV	GGLKGNVSI	AAGVTIENAI	GGSGNDVIVGNAANNVLKGGAGNDV	LFG	360 SMP (1SAT)
301	QRINLNEKSFSDV	GGLKGNVSI	AAGVTIENAI	GGSGNDVIVGNAANNVLKGGAGNDV	LFG	360 Serratia protease (1SRP)
				408	412	415
361	GGGADELWGGAGK	DIFV	FAASDSAPGASDWIRDFQKIDKIDLSFF	KEANSSFIHFV	420	Serralyisin (5D7W)
361	GGGADELWGGAGK	DIFV	FAASDSAPGASDWIRDFQKIDKIDLSFF	KEANSSFIHFV	420	SMP (1SAT)
361	GGGADELWGGAGK	DIFV	FAASDSAPGASDWIRDFQKIDKIDLSFF	KEANSSFIHFV	420	Serratia protease (1SRP)
	426	438	451			
421	DHFSGAGEALLSYNAS	NVTDLSVNIGGH	APDFLVKIVGQVDVATDFIV	471		Serralyisin (5D7W)
421	DHFSGAGEALLSYNAS	NVTDLSVNIGGH	APDFLVKIVGQVDVATDFIV	471		SMP (1SAT)
421	DHFSGAGEALLSYNAS	NVTDLSVNIGGH	APDFLVKIVGQVDVATDFIV	471		Serratia protease (1SRP)

Figure 2

Amino-acid sequence alignment of the serralysins from *Serratia* sp. FS14 (PDB entry 5d7w), *S. marcescens* (SMP; PDB entry 1sat) and *Serratia* sp. E-15 (*Serratia* protease; PDB entry 1srp). Identical residues are shown on a white background and differences are highlighted in red.

Figure 3

(a) Comparison of the serralysins from *Serratia* sp. FS14 (PDB entry 5d7w; magenta), *S. marcescens* (PDB entry 1sat; green) and *Serratia* sp. E-15 (PDB entry 1srp; grey). The significant distinct positions are magnified. (b) The electron density of the 189–198 loop of the serralysin from *Serratia* sp. FS14 showed that this loop was ordered in the structure. Structural superimpositions were performed with *PyMOL*.

Table 3

Distances between the Ca^{2+} ions in the parallel β -roll of the serralysin family and their ligands.

	Distance ($\text{\AA}$)		
	Serralysin	SMP	<i>Serratia</i> protease
Distance between Ca1 and			
Arg253 O	2.29	2.30	2.28
Gly255 O	2.37	2.36	2.46
Thr257 O	2.46	2.36	2.46
Asp285 OD1	2.46	2.55	2.48
Asp285 OD2	2.53	2.56	2.60
Gly287 O	2.30	2.31	2.38
Asp290 OD1	2.31	2.41	2.31
Distance between Ca2 and			
Gly288 O	2.33	2.38	2.49
Asp290 OD2	2.37	2.44	2.47
Thr327 O	2.34	2.36	2.52
Glu329 OE1	2.48	2.62	2.55
Glu329 OE2	2.46	2.58	2.74
Wat543/72/550	2.42	2.40	2.55
Wat541/42/524	2.36	2.35	2.67
Distance between Ca3 and			
Gly334 O	2.34	2.33	2.28
Gly336 O	2.36	2.38	2.53
Asp338 OD2	2.37	2.50	2.61
Gly351 O	2.33	2.32	2.51
Ala353 O	2.31	2.38	2.40
Asp356 OD1	2.40	2.47	2.38
Distance between Ca4 and			
Asn343 O	2.35	2.31	2.43
Ala345 O	2.32	2.36	2.51
Asn347 ND1	2.35	2.39	2.53
Gly360 O	2.33	2.40	2.49
Gly362 O	2.28	2.33	2.39
Asp365 OD1	2.41	2.49	2.63
Distance between Ca5 and			
Gly352 O	2.42	2.32	2.39
Gly354 O	2.39	2.43	2.62
Asp356 OD2	2.35	2.46	2.41
Gly369 O	2.38	2.40	2.50
Ala371 O	2.29	2.27	2.32
Asp374 OD1	2.38	2.48	2.59
Distance between Ca6 and			
Gly361 O	2.32	2.40	2.51
Gly363 O	2.40	2.35	2.51
Asp365 OD2	2.26	2.40	2.19
Asp383 O	2.21	2.20	2.25
Asp390 OD1	2.42	2.54	2.51
Wat173/561/604	2.72	2.81	2.72
Distance between Ca7 and			
Gly370 O	2.43	2.47	3.22
Gly372 O	2.38	2.46	2.65
Asp374 OD2	2.24	2.44	2.52
Asp400 OD1	2.55	2.55	2.60
Asp400 OD2	—	—	3.17
Wat99/629/—	2.37	2.29	—
Wat39/566/527	2.78	2.66	3.18

whole protein chain ($13.37 \text{ \AA}^2$) (labelled 2 in Fig. 3a; Fig. 3b). The serralysin reported here (Fig. 3a, magenta) has a wider active-site cavity and shows a more open conformation than SMP (Fig. 3a, green) and *Serratia* protease (Fig. 3a, grey). A previous study showed that this loop is disordered in the crystal structures of the free proteases but becomes ordered upon ligand binding. Asn191 in this loop in SMP moves $3.6 \text{ \AA}$ to make hydrogen bonds to the P3 residue of the ligand to close the entrance to the active cleft. Asn191 in serralysin from *Serratia* sp. FS14 moves more than $9.0 \text{ \AA}$ towards the molecular surface compared with SMP, and the movement of the C^α

atom of Asn191 may restrict the substrate or ligand specificity. The more open active-site cleft and entrance of serralysin reported here may be responsible for the substrate specificity of *Serratia* sp. FS14 serralysin.

Other than the above significant distinctions, another difference is observed in the zinc ligation. Similar to its homologues, the active-site zinc ion of serralysin from *Serratia* sp. FS14 is positioned at the bottom of the active-site cleft by five zinc ligands arranged in a distorted trigonal bipyramidal geometry, but the distance between the zinc and the hydroxyl O atom of Tyr216 is $2.66 \text{ \AA}$, which makes the coordinate bond more strong than that in its homologues ($2.75 \text{ \AA}$ in SMP and $3.05 \text{ \AA}$ in *Serratia* protease; Fig. 4). This may make the active site more stable in *Serratia* sp. FS14 serralysin than in the other serralysins, resulting in the thermostability of the protease.

Furthermore, as a result of the very high resolution, a few distinct differences are located in the β -strand sandwich domain. This domain mainly consists of 16 β -strands and is subdivided into two regions: an N-terminal region consisting of 13 β -strands ($\beta 6$ – $\beta 18$) and a C-terminal region consisting of three antiparallel β -sheets ($\beta 19$ – $\beta 21$). In the N-terminal region of SMP or *Serratia* protease, each of the β -strands is formed by only three amino-acid residues in an untwisted manner. However, in that of serralysin from *Serratia* sp. FS14 the β -sheets (including $\beta 8$, $\beta 9$, $\beta 10$, $\beta 11$, $\beta 13$, $\beta 14$, $\beta 15$, $\beta 17$, $\beta 19$, $\beta 20$ and $\beta 21$) are longer and are formed by four or five amino-acid residues; in particular, $\beta 8$, $\beta 10$ and $\beta 17$ are formed in a more twisted manner (Fig. 5). A characteristic of serralysin and its homologues is the presence of multiple calcium ions binding in the RTX domain; the binding of calcium ions facilitates the folding of serralysins and stabilizes the protein (Baumann, 1994; Baumann *et al.*, 1993). Comparison of the distances between the calcium ions and their coordinating ligands revealed that the binding affinity for calcium ions in *Serratia* sp. FS14 serralysin is relatively lower than that in the other two *Serratia* serralysins; this difference may also contribute to the thermostability of our serralysin (Table 3).

The crystal structure of serralysin from *Serratia* sp. FS14 represents the highest resolution serralysin-family structure

Figure 4

The active site of *Serratia* sp. FS14 serralysin. The zinc ion is coordinated by His176, His180, His186, Tyr216 and a water molecule, forming a distorted trigonal bipyramidal catalytic active centre.

Figure 5

Comparison of the 'parallel β -roll' C-terminal domains of serralyisin from *Serratia* sp. FS14 (PDB entry 5d7w; magenta), *S. marcescens* (PDB entry 1sat; green) and *Serratia* sp. E-15 (PDB entry 1srp; grey). The β -sheets in the C-terminal domain are labelled.

available to date and will be used as a model providing detailed information for future studies.

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References

- Adams, P. D. *et al.* (2010). *Acta Cryst.* **D66**, 213–221.
- Aiyappa, P. & Harris, J. (1976). *Mol. Cell. Biochem.* **13**, 95–100.
- Baumann, U. (1994). *J. Mol. Biol.* **242**, 244–251.
- Baumann, U., Bauer, M., Létoffé, S., Delepelaire, P. & Wandersman, C. (1995). *J. Mol. Biol.* **248**, 653–661.
- Baumann, U., Wu, S., Flaherty, K. M. & McKay, D. B. (1993). *EMBO J.* **12**, 3357–3364.
- Berman, H. M., Westbrook, J., Feng, Z., Gilliland, G., Bhat, T., Weissig, H., Shindyalov, I. N. & Bourne, P. E. (2000). *Nucleic Acids Res.* **28**, 235–242.
- Braun, V. & Schmitz, G. (1980). *Arch. Microbiol.* **124**, 55–61.
- Braunagel, S. C. & Benedik, M. J. (1990). *Mol. Gen. Genet.* **222**, 446–451.
- Chen, Y.-C., Shipley, G. L., Ball, T. K. & Benedik, M. J. (1992). *Mol. Microbiol.* **6**, 643–651.
- Daborn, P. J., Waterfield, N., Blight, M. A. & Ffrench-Constant, R. H. (2001). *J. Bacteriol.* **183**, 5834–5839.
- Delepelaire, P. & Wandersman, C. (1989). *J. Biol. Chem.* **264**, 9083–9089.
- Emsley, P., Lohkamp, B., Scott, W. G. & Cowtan, K. (2010). *Acta Cryst.* **D66**, 486–501.
- Givskov, M., Olsen, L. & Molin, S. (1988). *J. Bacteriol.* **170**, 5855–5862.
- Hamada, K., Hata, Y., Katsuya, Y., Hiramatsu, H., Fujiwara, T. & Katsube, Y. (1996). *J. Biochem.* **119**, 844–851.
- Kabsch, W. (2010). *Acta Cryst.* **D66**, 125–132.
- Kida, Y., Inoue, H., Shimizu, T. & Kuwano, K. (2007). *Infect. Immun.* **75**, 164–174.
- Lakshmi Bhargavi, P. & Prakasham, R. (2013). *Int. J. Biol. Macromol.* **61**, 479–486.
- Liang, T.-W., Kuo, Y.-H., Wu, P.-C., Wang, C.-L., Dzung, N. A. & Wang, S.-L. (2010). *J. Chin. Chem. Soc.* **57**, 857–863.
- Marty, K. B., Williams, C. L., Guynn, L. J., Benedik, M. J. & Blanke, S. R. (2002). *Infect. Immun.* **70**, 1121–1128.
- McCoy, A. J., Grosse-Kunstleve, R. W., Adams, P. D., Winn, M. D., Storoni, L. C. & Read, R. J. (2007). *J. Appl. Cryst.* **40**, 658–674.
- Miyata, K., Maejima, K., Tomoda, K. & Isono, M. (1970). *Agric. Biol. Chem.* **34**, 310–318.
- Miyata, K., Tomoda, K. & Isono, M. (1971). *Agric. Biol. Chem.* **35**, 460–467.
- Monreal, J. & Reese, E. T. (1969). *Can. J. Microbiol.* **15**, 689–696.
- Moriyama, K. & Okuda, K. (1992). *Seikagaku*, **64**, 149–159.
- Murshudov, G. N., Skubák, P., Lebedev, A. A., Pannu, N. S., Steiner, R. A., Nicholls, R. A., Winn, M. D., Long, F. & Vagin, A. A. (2011). *Acta Cryst.* **D67**, 355–367.
- Petersen, L. M. & Tisa, L. S. (2014). *J. Bacteriol.* **196**, 3923–3936.
- Pineda-Castellanos, M. L., Rodríguez-Segura, Z., Villalobos, F. J., Hernández, L., Lina, L. & Nuñez-Valdez, M. E. (2015). *Pathogens*, **4**, 210–228.
- Saleemuddin, M., Ahmad, H. & Husain, A. (1980). *Anal. Biochem.* **105**, 202–206.
- Sarker, M. R. & Cornelis, G. R. (1997). *Mol. Microbiol.* **23**, 410–411.
- Shanks, R. M., Stella, N. A., Hunt, K. M., Brothers, K. M., Zhang, L. & Thibodeau, P. H. (2015). *Infect. Immun.* **83**, 2907–2916.
- Zhang, L., Morrison, A. J. & Thibodeau, P. H. (2015). *PLoS One*, **10**, e0138419.
- Zheng, C.-C., Wang, C.-L., Fu, L., Wang, W.-W. & Xu, D.-Q. (2011). *Microbiology/Weishengwuxue Tongbao*, **38**, 228–236.