Acta Crystallographica Section F
**Structural Biology and Crystallization Communications**
ISSN 1744-3091

# Cloning, purification, crystallization and preliminary X-ray analysis of the *Burkholderia pseudomallei* L1 ribosomal protein

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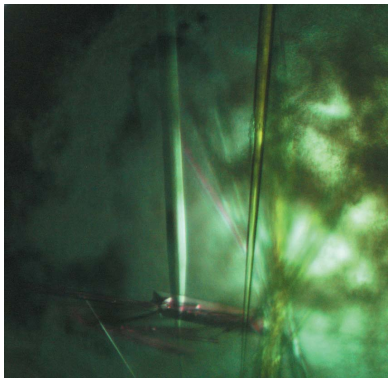
Received 10 January 2012
Accepted 3 February 2012





The gene encoding the L1 ribosomal protein from *Burkholderia pseudomallei* strain D286 has been cloned into the pETBLUE-1 vector system, overexpressed in *Escherichia coli* and purified. Crystals of the native protein were grown by the hanging-drop vapour-diffusion technique using PEG 3350 as a precipitant and diffracted to beyond 1.65 Å resolution. The crystals belonged to space group $P2_12_12$, with unit-cell parameters $a = 53.6$, $b = 127.1$, $c = 31.8$ Å and with a single molecule in the asymmetric unit.

## 1. Introduction

*Burkholderia pseudomallei* is a Gram-negative, aerobic, motile rod-shaped bacterium that thrives in hot wet soil and muddy water (Thibault *et al.*, 2004; Limmathurotsakul *et al.*, 2010). This microbe is the causative agent of melioidosis, a disease endemic in Southeast Asia and northern Australia (Currie *et al.*, 2008) that affects both man and animals (Dance, 2000; Titball *et al.*, 2008). The mode of infection is through ingestion, inhalation or wound contact with contaminated soil or water (Wiersinga *et al.*, 2006), and the severity of infection correlates with the size of the inoculum (Bondi & Goldberg, 2008). Following infection, the disease is manifested by a complex spectrum of symptoms, ranging from soft tissue, skeletal or organ abcesses (Wiersinga *et al.*, 2006) to pneumonia and fatal septicaemia (Chaowagul *et al.*, 1989; Beeker *et al.*, 1999; Currie, 2003). These symptoms mimic other common diseases such as tuberculosis, malaria and typhoid fever (Ip *et al.*, 1995), making diagnosis problematic and hampering treatment in a timely fashion, a problem which has been further exacerbated by a significant increase in antibiotic resistance (Sarkar-Tyson & Titball, 2010). A particular problem with melioidosis is that the pathogen can remain latent in the infected host for many years before symptoms emerge (Ngauy *et al.*, 2005).

To date, a number of *B. pseudomallei* genomes from a range of strains have been fully sequenced, including that of the most well characterized strain, K96243 (Holden *et al.*, 2004). The genome of the latter comprises two GC-rich chromosomes of 4.07 and 3.17 megabases, respectively (Holden *et al.*, 2004). Comparative genomic analysis using an *in silico* approach led to the identification of a set of 312 putative essential genes in *B. pseudomallei* that could form attractive targets for drug discovery (Chong *et al.*, 2006). As part of a focused programme on structural genomics, we have embarked on the structure determination of some of these proteins to facilitate the development of novel antibiotics.

The ribosome has long been recognized as an attractive target for antibiotic discovery (Hermann, 2005) and numerous antibiotics have been identified that interfere with its function in different ways, including competing with substrate binding, interfering with ribosomal dynamics, minimizing ribosomal mobility, facilitating miscoding, hampering the progression of the mRNA chain and blocking the nascent protein-exit tunnel (Yonath, 2005). The L1 subunit of the ribosome is located opposite the L7/L12 stalk and is one of the largest proteins in the 50S particle (Nevskaya *et al.*, 2005). Conformational changes in L1 are thought to be important in RNA binding and ribosome function (Nevskaya *et al.*, 2000). Biochemical studies have suggested that L1 plays an important role in ejecting deacylated tRNA from the ribosome (Nevskaya *et al.*, 2006). Whilst the structure

of L1 from a range of microbes, including *Thermus thermophilus* (Nevskaya *et al.*, 2006), *Methanococcus jannaschii* (Nevskaya *et al.*, 2000) and *M. thermolithotrophicus* (Nevskaya *et al.*, 2002), has been determined, the structure of the *B. pseudomallei* L1, a protein of 232 amino-acid residues, has not been reported. In this paper, we describe the cloning, overexpression, purification, crystallization and preliminary X-ray analysis of L1 ribosomal protein (BPSL3224) from *B. pseudomallei* strain D286.

## 2. Materials and methods

### 2.1. Cloning and overexpression

Genomic DNA was purified from *B. pseudomallei* strain D286, a pathogenic strain isolated from a melioidosis patient at Kuala Lumpur General Hospital (Lee *et al.*, 2007). The gene encoding *B. pseudomallei* L1 (*bpsl3224*) was PCR-amplified with the primers 5′-ATG GCC AAG ATC TCG AAG-3′ (forward) and 5′-AAT ACT TCG CTT CTT ACT GCG CG-3′ (reverse), designed on the basis of the sequence of *B. pseudomallei* K96243 (Holden *et al.*, 2004). To overcome possible amplification problems associated with the GC-rich nature of the *B. pseudomallei* DNA, 5% dimethylsulfoxide (DMSO) was included in the PCR mixture (Chakrabarti & Schutt, 2001). The purified DNA fragments were inserted into the pETBLUE-1 vector using the AccepTor vector kit (Novagen). Positive transformants were identified by blue/white screening and colony PCR. Plasmids were isolated and the gene sequence was identical to that of *bpsl3224* of strain K96243.

The plasmids were transformed into *Escherichia coli* Tuner (DE3) pLacI (Novagen) for overexpression. In order to produce sufficient protein for structural studies, a single colony of the transformed strain was used to inoculate a 250 ml flask containing 50 ml Luria–Bertani (LB) broth supplemented with 50 µg ml$^{-1}$ carbenicillin and 34 µg ml$^{-1}$ chloramphenicol and was grown overnight at 310 K at 250 rev min$^{-1}$. 5 ml of the overnight culture was used to inoculate 500 ml LB broth in 2 l flasks each supplemented as above. The flasks were incubated at 310 K until an $OD_{600}$ of 0.6 was reached, at which point overexpression was induced by the addition of 1 m*M* IPTG and the culture was grown for a further 4 h. The cells were harvested by centrifugation at 18 600*g* at 277 K (JLA-10.500 rotor, Avanti J-25I, Beckman) for 15 min and stored at 253 K.

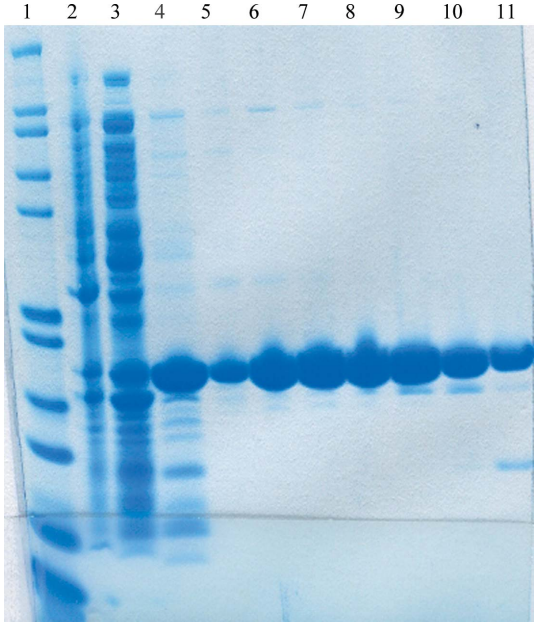



**Figure 1**
SDS–PAGE gel (NuPAGE 4–12% bis-tris gel, Invitrogen) showing stages in the purification of recombinant *B. pseudomallei* L1. Lane 1, Mark12 molecular-weight markers (Invitrogen); lane 2, cell debris after sonication; lane 3, supernatant after sonication; lane 4, pooled fractions from HiTrap SP-HP column; lanes 5–11, elution profile of L1 from the RESOURCE S column.

### 2.2. Purification

As *B. pseudomallei* L1 has an estimated isoelectric point of 9.7, a purification strategy based on cation-exchange column chromatography was adopted. Cell pellets were defrosted, suspended in about eight volumes of 50 m*M* MES buffer pH 6.5 and disrupted by sonication using a Soniprep 150 machine set at 16 µm amplitude for two cycles of 20 s each. Cell debris was removed by centrifugation at 70 000*g* for 10 min (JA-25.50 rotor, Avanti J25I, Beckman). The supernatant fraction was loaded onto a 5 ml HiTrap SP-HP column (GE Healthcare) and proteins were eluted with 75 ml of a linear gradient of 0.2–0.7 *M* NaCl in 50 m*M* MES buffer pH 6.5. L1 eluted at approximately 0.45 *M* NaCl and the pooled fractions were diluted threefold with 50 m*M* MES buffer pH 6.5. The fractions containing L1 were loaded onto a 1 ml RESOURCE S column (GE Healthcare) and proteins were eluted with 20 ml of a linear gradient of 0.1–0.5 *M* NaCl in 50 m*M* HEPES buffer pH 7.5. L1 eluted from the column at approximately 0.25 *M* NaCl and fractions containing L1 were pooled. Fig. 1 shows SDS–PAGE separation of the 24 kDa L1 protein after different stages of purification.

### 2.3. Crystallization of *B. pseudomallei* L1

Prior to crystallization, the pooled sample of purified L1 was concentrated to 18 mg ml$^{-1}$ using a Vivaspin centrifugal concentrator fitted with a 10 kDa molecular-weight cutoff filter (Sartorius). The buffer was exchanged to 10 m*M* Tris using a 0.5 ml Zeba Spin Desalting Column (Pierce). Preliminary crystallization conditions for L1 protein were screened using NeXtal suites (JCSG+, PACT and PEGs Suites from Qiagen) with a sitting-drop vapour-diffusion strategy on a Matrix Hydra II Plus One robot (Thermo Fisher Scientific, USA) by adding 0.2 µl of the L1 protein to the same volume of precipitant and then equilibrating against a 40 µl reservoir of the same precipitant at 289 K. Initial crystals were observed after 5 d of incubation under conditions with 0.2 *M* trilithium citrate and 20%(*w*/*v*) PEG 3350 as the precipitant. Optimization of these conditions using the hanging-drop vapour-diffusion technique with

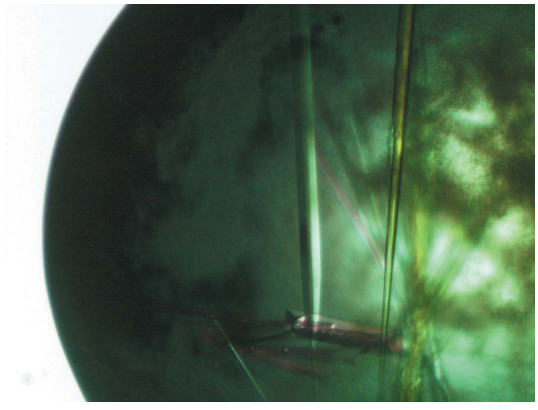


**Figure 2**
Crystals of *B. pseudomallei* L1 grown using 18%(*w*/*v*) PEG 3350, 0.2 *M* trilithium citrate.

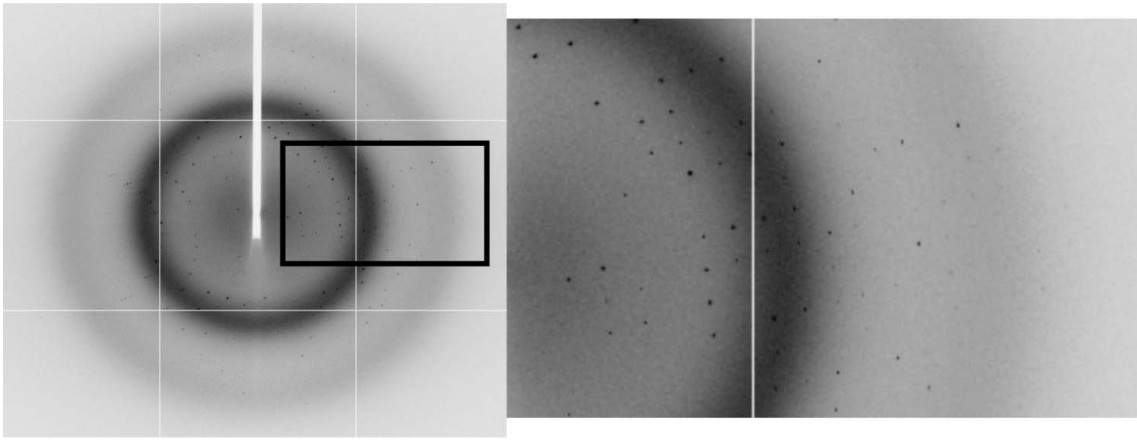


**Figure 3**
A representative 0.5° oscillation image of data collected from a *B. pseudomallei* L1 native crystal using an ADSC Q315 CCD detector on beamline I02 at Diamond Light Source, Oxford, England. An enlarged view of the region indicated by the square is shown on the right. The diffraction extends to the edge of the plate (1.65 Å).

**Table 1**
X-ray data-collection statistics for native crystals of *B. pseudomallei* L1.

Values in parentheses are for the highest resolution shell.

| | |
|---|---|
| Space group | $P2_12_12$ |
| Unit-cell parameters | |
| *a* (Å) | 53.6 |
| *b* (Å) | 127.1 |
| *c* (Å) | 31.8 |
| Temperature (K) | 100 |
| X-ray source | I02, Diamond Light Source |
| Detector | ADSC Q315 |
| Resolution (Å) | 33.23–1.65 (1.74–1.65) |
| Unique reflections | 26891 (3871) |
| $R_{\rm merge}$† (%) | 0.067 (0.456) |
| Completeness (%) | 99.5 (99.6) |
| Multiplicity | 4.6 (4.7) |
| Mean $I/\sigma(I)$ | 13.3 (3.3) |

† $R_{\rm merge} = \sum_{hkl}\sum_i |I_i(hkl) - \langle I(hkl)\rangle| / \sum_{hkl}\sum_i I_i(hkl)$, where $I_i(hkl)$ and $\langle I(hkl)\rangle$ are the observed intensity and the mean intensity of related reflections, respectively.

0.2 *M* trilithium citrate and 18%(*w*/*v*) PEG 3350 and a 2:1 ratio of protein to precipitant led to the growth of larger crystals with sharper edges (0.3 × 0.05 × 0.05 mm; Fig. 2).

### 2.4. Harvesting crystals and data collection

Crystals were flash-cooled to 100 K in a cryoprotectant solution made by adding 20% ethylene glycol to the optimized crystallization conditions. Crystals were tested for diffraction using an in-house Rigaku MicroMax-007 copper rotating-anode X-ray generator with Varimax confocal optics and a MAR Research image-plate desktop beamline. Data were subsequently collected from a native L1 crystal using a 80 µm beam on station I02 at the Diamond Light Source (250 images each of 0.5° oscillation to 1.65 Å resolution; Fig. 3) with an ADSC Q315 CCD detector. All images were collected at 60% transmission using 0.7 s exposure per image.

## 3. Results and discussion

Preliminary analysis of the diffraction data using the autoindexing routine in *iMOSFLM* (Leslie, 1992; Leslie & Powell, 2007; Battye *et al.*, 2011) showed that the native L1 crystals belonged to a primitive orthorhombic space group with unit-cell parameters $a = 53.6$, $b = 127.1$, $c = 31.8$ Å. This suggested that the asymmetric unit must contain a single subunit with a Matthews coefficient of 2.23 Å$^3$ Da$^{-1}$ (45% solvent content), as all other possible values of $V_{\rm M}$ for multiple subunits in the asymmetric unit lay outside the allowed range (Matthews, 1976). The native L1 data set was processed using *iMOSFLM* and *SCALA* (Winn *et al.*, 2011). Analysis of the pattern of systematic absences was consistent with the crystal being assigned to space group $P2_12_12$. Data-collection and processing statistics are shown in Table 1. It is hoped that the structure of the L1 ribosomal protein can be solved using MAD, and attempts to prepare selenomethionine-substituted crystals are currently under way in the hope of achieving a better understanding of the function of L1.

RM, SN and DWR acknowledge the Ministry of Science, Technology and Innovation, Government of Malaysia (grant 07-05-16-MGI-GMB08) and the British Council PMI-2 Initiative for financial support. We acknowledge the Diamond Synchrotron for provision of beamtime and thank James Sandy for assistance with station I02. AGAA thanks Universiti Putra Malaysia and the Ministry of Higher Education Malaysia for scholarship funding.

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