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Crystallization and preliminary crystallographic analysis of LipC12, a true lipase isolated through a metagenomics approach

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LipC12, a true lipase from family I.1 of bacterial lipases which was previously isolated through a metagenomics approach, contains 293 amino acids. Among lipases of known three-dimensional structure, it has a sequence identity of 47% to the lipase from *Pseudomonas aeruginosa* PAO1. Recombinant N-terminally His_6-tagged LipC12 protein was expressed in *Escherichia coli*, purified in a homogenous form and crystallized in several conditions, with the best crystals being obtained using 2.0 *M* sodium formate and 0.1 *M* bis-tris propane pH 7.0. X-ray diffraction data were collected to 2.70 Å resolution. The crystals belonged to the tetragonal space group $P4_122$, with unit-cell parameters $a = b = 58.62$, $c = 192.60$ Å.

1. Introduction

Different approaches have been used in the search for novel industrial enzymes. These include directed evolution (Nakagawa *et al.*, 2007) and rational design (Kazlauskas & Bornscheuer, 2009) of known lipases, the isolation of new lipase-producing microorganisms and the construction and screening of metagenomic libraries (Suenaga, 2011; Glogauer *et al.*, 2011; Couto *et al.*, 2010; Hårdeman & Sjöling, 2007; Usami *et al.*, 2003; Marhuenda-Egea & Bonete, 2002).

Metagenomic libraries are collections of clones produced from fragments of DNA extracted from certain environments. It is possible to express new genes from the library and to produce new enzymes coded by these genes without the need to isolate and culture the organism from which the gene is derived. Various new lipases and esterases have been found using metagenomic libraries (Kim *et al.*, 2010; Jeon *et al.*, 2009; Elend *et al.*, 2007; Rhee *et al.*, 2005).

Recently, we reported the isolation and characterization of LipC12 (Glogauer *et al.*, 2011; GenBank JF417979) obtained from the metagenome of fat-contaminated soil from the bank of an anaerobic lagoon of the waste-water treatment plant of a meat-packing and dairy industry located in Carambeí, Paraná State, Brazil. This lipase has a sequence identity of 72% to the putative lipases from *Yersinia* spp. (GenBank CBY26912 and YE1842). Compared with lipases of known three-dimensional structure, LipC12 possesses 47 and 41% sequence identity to those from *Pseudomonas aeruginosa* (PDB entry 1ex9; Nardini *et al.*, 2000) and *Burkholderia cepacia* (PDB entry 1oil; Kim *et al.*, 1997), respectively. It belongs to family I.1 of bacterial lipases, has chaperone-free folding, does not possess disulfide bridges and is calcium-ion-dependent. A preliminary evaluation of its biotechnological potential was carried out and the most notable results of the characterization studies were a high specific activity towards long-chain triacylglycerols, activity and stability over a wide range of pH values, good thermal stability, and stability in water-miscible organic solvents and at high salt concentrations (Glogauer *et al.*, 2011). These characteristics suggest that this lipase has the potential to perform well in biocatalytic processes such as the production of biodiesel through transesterification in organic media.

In the current paper, we report the crystallization and preliminary crystallographic characterization of LipC12. This work provides a basis for further studies to determine the three-dimensional structure of LipC12. Once the three-dimensional structure is known, it will be

Table 1
Data-collection and processing statistics.

Values in parentheses are for the highest resolution shell.

No. of crystals	1
X-ray source	MicroMax-007 rotating-anode generator
Wavelength (Å)	1.5418
Detector	R-AXIS IV^{++}
Temperature of data collection (K)	100
Crystal-to-detector distance (mm)	179.82
Rotation range per image (°)	1
Total rotation range (°)	180
Exposure time per image (s)	1200
Resolution range (Å)	193–2.70 (2.78–2.70)
Space group	$P4_122$
Unit-cell parameters (Å)	$a = b = 58.62$, $c = 192.60$
Mosaicity (°)	0.12
Total No. of measured intensities	127086 (9460)
Unique reflections	9471 (699)
Multiplicity	3.5 (3.3)
Mean $I/\sigma(I)$	24.27 (5.17)
Completeness (%)	95.5 (85.5)
R_{merge}† (%)	7.2 (40.4)
R_{meas} (%)	13.3 (57.2)
Overall B factor from Wilson plot (Å^2)	47.3

† $R_{merge} = \sum_{hkl}\sum_i |I_i(hkl) - \langle I(hkl)\rangle| / \sum_{hkl}\sum_i I_i(hkl)$, where $I_i(hkl)$ is the ith observation of reflection hkl and $\langle I(hkl)\rangle$ is the average intensity for all observations i of reflection hkl.

possible to plan specific mutations with the intention of improving the stability and activity of this promising lipase under the harsh reaction conditions that are encountered in transesterification processes.

2. Experimental procedures

2.1. Protein expression and purification

The isolation, molecular cloning and purification of LipC12 have been described by Glogauer *et al.* (2011). A single affinity-chromatography step was sufficient to purify the protein, as revealed by SDS–PAGE, giving a yield of 71 mg purified N-terminally His_6-tagged protein per litre of culture medium. Without any further processing, the protein was concentrated to 10 and 20 mg ml^{-1} in 50 m*M* Tris–HCl, 150 m*M* NaCl, 2 m*M* $CaCl_2$, 10%(*v*/*v*) glycerol using a Vivaspin 6 device (GE Healthcare). Protein concentration was determined using the Bradford assay (Bradford, 1976).

2.2. Crystallization

Initial crystallization trials were performed using the sitting-drop vapour-diffusion method (McPherson, 1999) with a Genomic Solutions Cartesian (Digilab Genomic Solutions) robot at the Physics Institute of São Carlos, São Paulo University and the following kits: PEGs Suite, Classics Suite, Classics II Suite (from Qiagen), SaltRx and Index (from Hampton Research). Each drop was prepared by mixing 1 µl reservoir solution and 1 µl protein solution at three different concentrations: 5, 10 and 20 mg ml^{-1}. The drops were then equilibrated against 100 µl reservoir solution in 96-well plates at 291 K.

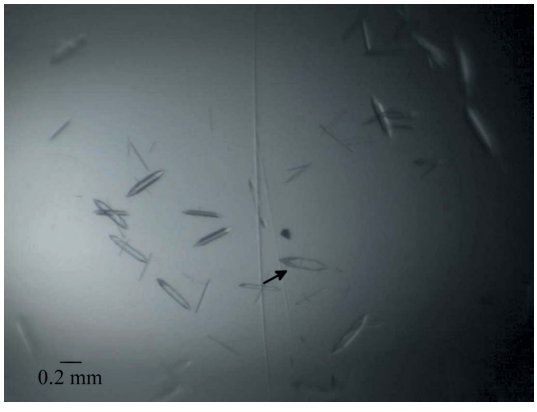

Figure 1
Crystals of LipC12 grown in a solution consisting of 2.0 *M* sodium formate and 0.1 *M* bis-tris propane pH 7.0. An arrow indicates the (large) crystal that gave the best data set, with approximate dimensions of 0.5 × 0.1 × 0.1 mm. Other data-collection attempts showed that the needles in the drops have the same unit cell.

2.3. X-ray data collection and processing

Crystals were transferred to a cryoprotectant solution, which consisted of the original precipitant containing 20%(*v*/*v*) ethylene glycol, just before being flash-cooled at 100 K in a cold nitrogen stream. X-ray diffraction experiments were performed with a MicroMax-007 rotating-anode generator and an R-AXIS IV^{++} (Rigaku) detector at the Physics Institute of São Carlos, São Paulo University. Indexing, integration and scaling were performed with the *XDS* suite (Kabsch, 2010).

3. Results and discussion

LipC12 was overexpressed and purified for structural analyses. Crystals formed in several of the crystallization kits under very different conditions. In almost all cases the crystals grew as numerous tiny needles which were visible under a microscope on the following day. Some of the conditions produced thicker crystals, with the best condition being 2.0 *M* sodium formate and 0.1 *M* bis-tris propane pH 7.0 using a protein concentration of 10 mg ml^{-1} (Fig. 1). After growth for ten weeks, these conditions allowed the collection of a complete data set to 2.70 Å resolution at 100 K (Fig. 2) with an overall R_{merge} of 7.2%. The LipC12 crystals belonged to the tetragonal space group $P4_122$, with unit-cell parameters $a = b = 58.62$, $c = 192.60$ Å. Other statistical data are shown in Table 1. Based on a molecular mass of 33 395 Da for the His-tagged LipC12 monomer (20 expression residues added to the 293 native residues), the crystal-

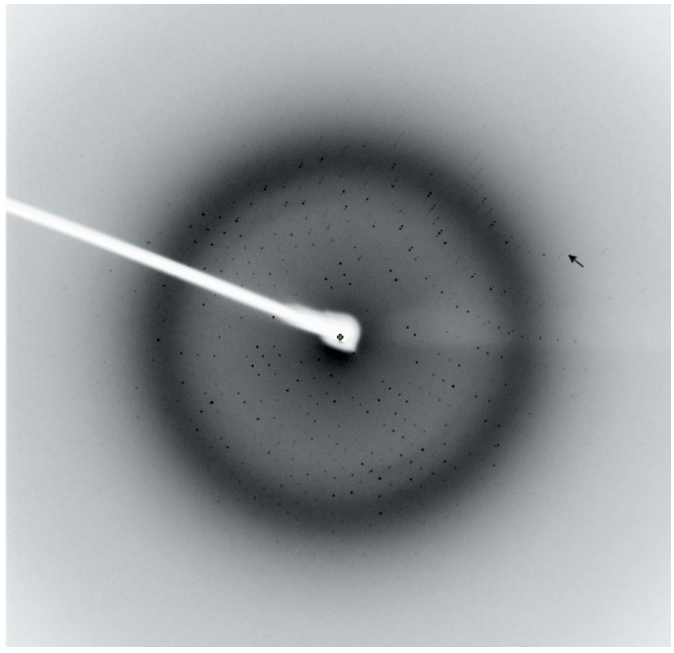

Figure 2
Diffraction image of the crystal in Fig. 1. The arrow denotes the 2.7 Å diffraction limit.

packing parameter (Matthews, 1968) is 2.48 Å^3 Da^{-1} and the estimated solvent content is 50.4% for one monomer in the asymmetric unit. To obtain phase information, molecular replacement was performed using an edited model of the structure of *P. aeruginosa* lipase (47% sequence identity and 73% sequence similarity; PDB entry 1ex9; Nardini *et al.*, 2000) as a template, which was prepared using the program *CHAINSAW* (Stein, 2008). A clear solution was found by the program *Phaser* (McCoy *et al.*, 2007). Trials to obtain better crystals are currently being undertaken in order to provide data that will allow refinement of this solution.

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