TITLE: GENE EXPRESSION, PROTEIN PURIFICATION AND CRYSTAL STRUCTURE OF L,D-TRANSPEPTIDASE 3 FROM *MYCOBACTERIUM TUBERCULOSIS*.

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ABSTRACT

Mycobacterial cell wall is a complex structure composes mainly by arabinogalactan, mycolic acids and peptidoglycan (PG). PG shows unique chemical modifications found frequently in *Mycobacterium*, such as a high percentage of inter-peptide non-classical *m*DAP-*m*DAP linkages, which are catalysed by L,d transpeptidases (Ldts), a group of conservate proteins which seem to have several functions. There are five paralogues encoding Ldts in *M. tuberculosis* of which Ldt_{Mt1}, Ldt_{Mt2} and Ldt_{Mt5} demonstrated to be essentials to the cell wall homeostasis; however, there are no genetic, functional or structural studies on $Ldt_{Mt3,}$ a putative L,d transpeptidase. This study aims to determine the crystal structure of Ldt_{Mt3} to correlate the enzyme structure with its possible function. Ldt_{Mt3} was cloned in pET28a vector, and overexpressed in BL21 (DE3). Protein was purified by Immobilized Metal Affinity Chromatography (IMAC) and then by Size Exclusion Chromatography (SEC). Crystallization was carried out and X-ray data set were obtained at LNLS-(Campinas-Brasil). We solved the structure by molecular replacement. Ldt_{Mt3} was successfully overexpressed in *E. coli*. Protein was extracted by sonication and purified by his-tag affinity and SEC and we have a yield of almost 2.5 mg of protein by liter of culture. The purity of the protein was checked by SDS-PAGE. Ldt_{Mt3} crystals were obtained after 48h by hanging drop vapor diffusion method and micro seeding in a reservoir containing 10% w/v PEG 8000, 100 mM Hepes pH 7.5, 200 mM calcium acetate. These crystals diffracted up to 1.61Å and belong to the spatial group $P2_12_12_{1.}$ Analysis of the structure shows two domains, an N-terminal Bacterial Immunoglobulin like Domain (BIgB) and a C-terminal Catalytic Domain (CD). Three pathways communicate to the Ldt_{Mt3} catalytic centre which contains catalytic residues often found in hydrolytic enzymes and transferases such as Cys246, His228 and Ser229. Structural comparison with other Ldts demonstrated high similarity with Ldt_{Mt1} (Cα RSMD 0.9 Å), and less similarity with Ldt_{Mt2} (Cα RSMD 1.9 Å) and Ldt_{Mt5} (Cα RSMD 2.1). In conclusion, the crystal structure of Ldt_{Mt3} reported here is according with a transpeptidase function; additionally, this crystallization protocol and structure could be useful to develop projects in drug discovery against Ldts from *Mycobacterium tuberculosis*.

Keywords: *Mycobacterium tuberculosis*, Peptidoglycan, L,d transpeptidases

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