STRUCTURE OF L,D TRANSPEPTIDASE-2 (Ldt_{Mt2}) FROM MYCOBACTERIUM TUBERCULOSIS REVEALS A PEPTIDOGLYCAN FRAGMENT BINDING IN THE ACTIVE SITE

Autors Libreros-Zúñiga G.A[1, 2,3], Bury P.[1] and Dias M.V.B[1].

Institutions [1.]Departamento de Microbiologia, Instituto de Ciências Biomédicas, Universidade de São Paulo (Avenida Prof. Lineu Prestes 1374-Butantã 05508-900) São Paulo, SP,Brasil. [2.] UNESP. Universidade Estadual Paulista (Rua Cristovão Colombo 2265-Jardim Nazareth-15.054.000 São Jose do Rio Preto, SP, Brasil. [3.]Departamento de Microbiología, Escuela de Ciencias Básicas, Universidad del Valle (Calle 4B 36-00 Barrio San Fernando) Cali, Colombia.

Summary:
The incidence of multiple drug resistant *Mycobacterium tuberculosis* is increasing worldwide, and it is necessary to develop new drugs against this disease. L,D-transpeptidases (Ldt_{Mt}) catalyse the peptidoglycan non-classical 3-3 transpeptide linkages and usually are not inhibited by β-lactams. Its association with an intrinsic β-lactamase prevents the use of these antibiotics to tuberculosis therapy. Disruption of Ldt_{Mt2} results in severe morphological and functional alterations; therefore, Ldt_{Mt2} is a validated target for drug discovery. This work aims to obtain the structure of Ldt_{Mt2} in complex with inhibitors and peptidoglycan fragment. Ldt_{Mt2} was amplified from *M. tuberculosis* H37Rv genomic DNA and cloned in pET28a, and overexpressed in BL21(DE3). Protein was purified by IMAC and SEC and crystallization was carried out. X-ray data set were obtained at LNLS-(Campinas-Brasil) and we solved the structure by molecular replacement.Currently, we successfully overexpressed and purified Ldt_{Mt2}. Protein purity was checked by SDS-PAGE. Ldt_{Mt2} crystals were obtained after 24-48h and they diffracted up to 2.3Å and belong to the spatial group $I_{212121.}$ The analysis of the structure shows a fragment of *E. coli* peptidoglycan extending to the active site and might prevent the binding of compounds. Preliminary analysis of thermal shift shows that meropenem causes a decreasing of T_m of Ldt_{Mt2} compared with apoenzyme. We believe that compound could dislocate this peptidoglycan fragment and consequently decrease the melting temperature. We have established the expression, purification and crystallization conditions for Ldt_{Mt2}. The presence of peptidoglycan in its active site indicates that a further purification step is needed to obtain apoenzyme, which is a requirement for structural based drug discovery. In the next step, we aim to screen a library of compounds through biophysical techniques, such as thermal shift and obtain protein-ligand complexes through co-crystallization or soaking. The hits obtained will be a start point for promising inhibitors against *Mycobacterium tuberculosis.*

Keywords
Tuberculosis, Ldt_{Mt2}, drug discovery.
Founding
FAPESP