TARGETING THE MYCOBACTERIUM TUBERCULOSIS CELL WALL: FRAGMENT BASED DRUG DISCOVERY AGAINST L,D TRANSPEPTIDASES 1 AND 2.

Libreros-Zúñiga G.A\textsuperscript{1,2,3} and Dias M.V.B\textsuperscript{1}

1. Departamento de Microbiologia, Instituto de Ciências Biomédicas, Universidade de São Paulo, São Paulo, Brazil.
2. IBILCE, Universidade Estadual Paulista, São Jose do Rio Preto, São Paulo, Brazil.
3. Departamento de Microbiología, Escuela de Ciencias Básicas, Universidad del Valle, Cali, Colombia.

INTRODUCTION
The emergence of multidrug resistant Mycobacterium tuberculosis (Mt) has led to the development of new anti-tuberculosis drugs. L,D-transpeptidases (Ldt\textsubscript{Mt}) catalyzes 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 treat tuberculosis. Ldt\textsubscript{Mt1} and Ldt\textsubscript{Mt2} are essential for Mt and their disruption results in severe morphological and functional alterations.

OBJECTIVES
To identify inhibitors for L,D-transpeptidases from Mycobacterium tuberculosis by Fragment Based Drug Discovery.

MATERIALS AND METHODS
Ldt\textsubscript{Mt1} and Ldt\textsubscript{Mt2} were cloned in pET vectors, and overexpressed in BL21(DE3). Proteins were 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. Thermal shift was performed using apoenzyme and in complex meropenem.

RESULTS
Ldt\textsubscript{Mt1} and Ldt\textsubscript{Mt2} were successfully overexpressed. Proteins were extracted by sonication and purified by his-tag affinity and SEC. The purity of the protein was checked by SDS-PAGE. Ldt\textsubscript{Mt2} crystals were obtained after 24-48h and they diffracted up to 2.3Å and belong to the spatial group I\textsubscript{2}12\textsubscript{1}2\textsubscript{1}2\textsubscript{1}. Analysis of the structure shows a fragment of peptidoglycan. This molecule extends to all 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\textsubscript{Mt2} compared with apoenzyme.

CONCLUSIONS
We have established the expression, purification and crystallization conditions for Ldt\textsubscript{Mt2}. The presence of peptidoglycan in the active site of Ldt\textsubscript{Mt2} indicates that a further purification step is needed to obtain apoenzyme, which is a requirement for fragment screening.

In the next step, we aim to screen a library of compounds through thermal shift and obtain protein-ligand complexes through co-crystallization or soaking. The hits obtained will be a start point for promising inhibitors against Mt.

ACKNOWLEDGEMENTS
FAPESP

KEY WORDS
Tuberculosis, L,d transpeptidases, drug discovery.