Abstract

Tuberculosis is a disease usually caused by the bacillus *Mycobacterium tuberculosis*, which is considered a global public health threat. The TB treatment currently recommended is long and involves undesirable side effects to the administered drugs, leading patients to give up the chemotherapy. Thus, it is essential the development of new drugs and vaccines to treat tuberculosis. Homoserine dehydrogenase is an attractive target for rational drug design. The *thrA* gene was amplified from *M. tuberculosis* H37Rv genomic DNA, cloned into the pCR®-Blunt vector and subcloned into the pET-23a(+) expression vector. The protein was expressed in the soluble fraction of BL21-Lys(DE3) *Escherichia coli* cells.

Introduction

Tuberculosis (TB) is a disease usually caused by the bacillus *M. tuberculosis*, which is considered a global public health threat. According to the world health organization, it was estimate that 9.27 million new cases of TB occurred in 2007 (WHO, 2009), causing around of two million deaths annually (WHO, 2006). The global incidence of TB is 137 cases per 100,000 population, where the highest estimated TB incidence rates are in African countries; that is linked to the high rates of HIV coinfection, poverty and lack of medicines (Corbett, 2003). The TB treatment currently recommended is long and involves undesirable side effects to the administered drugs, leading patients to give up the chemotherapy. Thus, the emergence of drug resistance especially multidrug-resistant (MDR-TB) and current extensively drug-resistant (XDR-TB) strains are usually caused by low patient's adherence to treatment (WHO, 2006).
Therefore, with the increase of TB incidence worldwide there is the need for the development of more effective and less toxic anti-tubercular agents. Likewise, the understanding of essential \textit{M. tuberculosis} metabolic routes as the aspartate pathway and the characterization of the enzymes involved in this pathway are an important step toward the development of new drugs and vaccines to treat TB in order to improve the current treatment. Homoserine dehydrogenase (HSD) catalyzes the third step of the aspartate pathway. This enzyme catalyzes the NADPH-dependent reduction of aspartate-4-semialdehyde into homoserine (Galili, 1995), which is the precursor of threonine, isoleucine and methionine. This enzyme appears to be the central point of regulatory mechanisms by the exhibit end-product allosteric inhibition by threonine (Galili, 1995; Dominique et al., 1993). HSD is not found in humans; thus threonine, isoleucine and methionine are obtained from the diet (Bareich et al., 2003; Jacques et al., 2001). HSD from \textit{M. tuberculosis} is therefore an attractive target for drug design due to the possibility to find a selective inhibitor to be used as anti-tubercular agent.

**Methodology**

The \textit{thrA} gene (Rv1294) encoding \textit{M. tuberculosis} HSD (EC 1.1.1.3) was identified by homology in the genome of \textit{M. tuberculosis} H37Rv (Cole et al., 1998). Two oligonucleotide primers complementary to regions 5’ and 3’ of \textit{thrA} gene were design to contain Ndel and HindIII restriction sites, respectively. The \textit{thrA} gene was amplified from the \textit{M. tuberculosis} H37Rv genomic DNA by Polymerase Chain Reaction (PCR). The PCR product in agreement with the expected size (1326 bp) was cloned into pCR®-Blunt vector (Invitrogen). Then, the \textit{thrA} gene was extracted from pCR®-Blunt vector using Ndel and HindIII restriction enzymes and subcloned into pET-23a(+) expression vector (Novagen). Automatic DNA sequencing was performed to confirm the identity of \textit{thrA} gene. Electrocompetent \textit{E. coli} strains were transformed with the recombinant plasmid (pET-23a(+)::\textit{thrA}) by electroporation. Different expression conditions were employed in order to obtain HSD expression in the soluble fraction. The protein expression was analyzed by SDS-PAGE.

**Results and discussion**

Automatic DNA sequencing confirmed both identity and integrity of \textit{thrA} gene of \textit{M. tuberculosis} H37Rv and the absence of PCR introduced mutations. The expression of HSD was observed in the insoluble fraction of Rosetta(DE3) \textit{E. coli} strains at 37°C, using the
culture medium LB, with isopropyl-β-D-thiogalactopyranoside (IPTG) induction and in the absence of IPTG. The protein was also expressed in the insoluble fraction of BL21-Lys(DE3) E. coli strains at 37°C, using as a culture medium TB or LB and with IPTG induction. However, a small amount of protein could be found in the soluble fraction of BL21-Lys(DE3) at 30°C, using as a culture medium TB, LB or 4YT and with IPTG induction, though the major amount of protein is yet expressed in the insoluble fraction.

Conclusions

The next step of this work will be the optimization of the HSD expression in the soluble fraction and the purification of the protein. Availability of homogeneous M. tuberculosis HSD protein will allow the biochemical and kinetic characterization of the enzyme which is important step toward the development of new drugs to treat TB.

References


