Recovery of recombinant Mycobacterium tuberculosis antigens fused with cell wall anchoring motif (LysM) from inclusion bodies using non-denaturing reagent (N-lauryl sarcosine)

ABSTRACT

Background The current limitations of conventional BCG vaccines highlights the importance in developing novel and effective vaccines against tuberculosis (TB). The utilization of probiotics such as Lactobacillus plantarum for the delivery of TB antigens through in-trans surface display provides an effective and safe vaccine approach against TB. Such non-recombinant probiotic surface display strategy involves the fusion of candidate proteins with cell wall binding domain such as LysM, which enables the fusion protein to anchor the L. plantarum cell wall externally, without the need for vector genetic modification. This approach requires sufficient production of these recombinant fusion proteins in cell factory such as Escherichia coli which has been shown to be effective in heterologous protein production for decades. However, over expression in E. coli expression system resulted in limited amount of soluble heterologous TB-LysM fusion protein, since most of it are accumulated as insoluble aggregates in inclusion bodies (IBs). Conventional methods of denaturation and renaturation for solubilizing IBs are costly, time-consuming and tedious. Thus, in this study, an alternative method for TB antigen-LysM protein solubilization from IBs based on the use of non-denaturating reagent N-lauroylsarcosine (NLS) was investigated. Results Expression of TB antigen-LysM fusion genes was conducted in Escherichia coli, but this resulted in IBs deposition in contrast to the expression of TB antigens only. This suggested that LysM fusion significantly altered solubility of the TB antigens produced in E. coli. The non-denaturing NLS technique was used and optimized to successfully solubilize and purify ~ 55% of the recombinant cell wall-anchoring TB antigen from the IBs. Functionality of the recovered protein was analyzed via immunofluorescence microscopy and whole cell ELISA which showed successful and stable cell wall binding to L. plantarum (up to 5 days). Conclusion The presented NLS purification strategy enables an efficient and rapid method for obtaining higher yields of soluble cell wall-anchoring Mycobacterium tuberculosis antigens-LysM fusion proteins from IBs in E. coli.

Keyword: Mycobacterium tuberculosis antigen; Lysine motif; Overexpression; Inclusionbody; TB subunit vaccine; N-lauroylsarcosine