Extended Abstract

Efficient method for enrichment of lipophilic proteins from mycobacterial diseases for two-dimensional gel electrophoresis

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Abstract:

Lipophilic proteome profiling is crucial because they have an anticipated role in biological processes and pathogenesis of Mycobacterial diseases. These lipophilic proteins might be used as potential targets due to their association with membranes and drugs. We developed an efficient and rapid method to enrich the lipophilic proteins extraction from *Mycobacterium tuberculosis* H37Rv for 2DE. In the extraction of lipophilic proteins, nonionic detergent was added in sonication buffer that augmented the solubilization of the proteins at the time of sonication. Enriched whole cell lysate was subjected to direct phase separation using Triton X-114, without the need for preisolation of membranes. In this study, we report that our optimized extraction buffer increased the lipophilic proteins extraction and their improved resolution on 2D gel up to two- to threefolds as compared to standard extraction buffer.

Introduction:

Tuberculosis (TB) is a global public health problem, which is the consequence of *Mycobacterium tuberculosis* infection. There are approximately 9 million new cases of TB and 1.5 million deaths [1]. Emergence of multidrug-resistant TB, extensively drug-resistant TB, and totally drug-resistant TB [2, 3] remains a global health problem that not only hinders the prevention or control but also the treatment of TB. Rapid identification of these drug-resistant strains is one of the most important strategies against the TB.

Lipophilic proteins have played an important role in the pathogenesis of M. tuberculosis and therefore the identification of lipophilic proteins are important in relation to resistance as it may help in developing novel diagnostic probes and drug targets [4–8]. Malen et al. [9] reported that Triton X-114 was an efficient method for extraction of lipophilic pro- Correspondence: JALMA Institute for Leprosy and Other Mycobacterial Diseases, Agra, India Abbreviation: MALDI-TOF/MS, Matrix Assisted Laser Desorption Ionization-Time of Flight/Mass Spectrometry; MDRTB, Multi drug-resistant TB; TB, tuberculosis; TDR-TB, Totally drug resistance TB; XDR-TB, Extensively drug resistant TB teins from mycobacteria, without the need for preisolation of membranes. 2DE is the best tool for expression proteomics of complex biological samples because not only due to its resolution and sensitivity but also protein species separation (as compared to bottom-up approaches, LC/MS) [10]. The analysis of lipophilic proteins by 2DE has been a tedious task not only due to difficulty in solubility of lipophilic proteins but also their relatively low abundance in a background of highly abundant cytoplasmic proteins. Lipophilic proteins solubility could be improved by using detergents and chaotropes. Severe quantitative loss of proteins is often observed in high resolution 2DE of lipophilic proteins. Therefore, it is necessary to improve the sample preparation procedures for 2DE of lipophilic proteins.

Materials and Methods:

Selection of isolate, culture, and growth harvesting: *Mycobacterium tuberculosis* H37Rv was collected from the Mycobacterial Repository Centre, NJIL & OMD, Agra, India. It was grown in Sauton's liquid medium for 4 wks at 37°C. After 4 wks culture was harvested.

Enrichment of lipophilic proteins and their phase separation: Whole cell lysate was prepared according to the published protocol with slight modification for enriching the lipophilic proteins [7]. Briefly, 1 g wet cells mass was washed with normal saline and then suspended in 5 mL sonication buffer (50 mM Tris–HCl containing 10 mM MgCl2, 0.1% sodium azide, 1 mM PMSF, Protease inhibitor cocktail (PIC) and 1 mM EGTA; pH 7.4) with Triton X-100 (1% v/v, Sigma) and then broken by intermittent sonication for 15 min at 4°C. To remove the cell debris homogenate was initially centrifuged at 2300 × g for 20 min at 4°C. Triton X-114 (Sigma) was added in whole cell lysate (final detergent concentration 2% v/v) and the suspension was stirred at 4°C for 20 min to obtain the protein extract in a single phase. Residual insoluble matter was

removed by centrifugation at 15 700 × g for 10 min, and the solution was separated in two phases, an upper (aqueous) and a lower (detergent) phase after 10 min incubation at 37°C. The detergent phase was collected and proteins were precipitated by 1:4 ratios of HPLC gradeacetone Sisco research laboratories (SRL). The protein pellet was washed and dissolved in 2D rehydration buffer. Protein concentration was estimated by Bradford method using BSA as standard. Protein extractions and quantifications were done in technical replicas.

Results and Discussion:

Approximately 480–500 protein spots were visualized in the 2D gel of normal Triton X-114 extraction protocol (Fig. 1A). In the 2D gel of our improved extraction protocol (Triton X-100 treatment followed by Triton X-114 extraction) we found approximately 1000–1050 protein spots (Fig. 1B). On comparison of 2D gels, we found the qualitative and quantitative differences

Table 2. Details of proteins identified by MS

Spot number	Accession number	Protein identified	MASCOT score	Nominal mass (Da)	p/	Sequence coverage %	ORF number	Functional category[a)]
D 1	P9WK13 (MDH_MYCTU)	Malate dehydrogenase	56	34 301	4.65	38	Rv1240	1
D 2	P9WQA5 (Y2971_MYCTU)	Uncharacterized oxidoreductase	92	30 345	4.70	51	Rv2971	1
D 3	P9WHF3 (RHO_MYCTU)	Transcription-terminating factor Rho	91	65 094	5.57	27	Rv1297	2
D 4	053872 (O53872_MYCTU)	3-hydroxyacyl-CoA dehydrogenase	90	76 072	5.42	26	Rv0860	3
D 5	P9WQ59 (FAA28_MYCTU)	Long-chain fatty acid AMP ligase FadD28	61	62 602	5.22	22	Rv2941	3
D 6	P9WGI9 (GLYA1_MYCTU)	Serine hydroxymethyl transferase 1	92	46 187	6.12	37	Rv1093	1
D 7	053665 (O53665_MYCTO)	Probable 3-oxoacyl-reductase FabG4	61	46 798	6.38	16	Rv0242c	3
D 8	P9WGR1 (INHA_MYCTU)	Enoyl-[acyl-carrier-protein] reductase	98	28 510	5.57	47	Rv1484	3
D 9	P9WNP1 (ECHA6_MYCTU)	Probable enoyl-CoA hydratase echA6	88	26 012	5.97	37	Rv0905	3

a) 1: intermediary metabolism and respiration; 2: information pathways; 3: lipid metabolism.

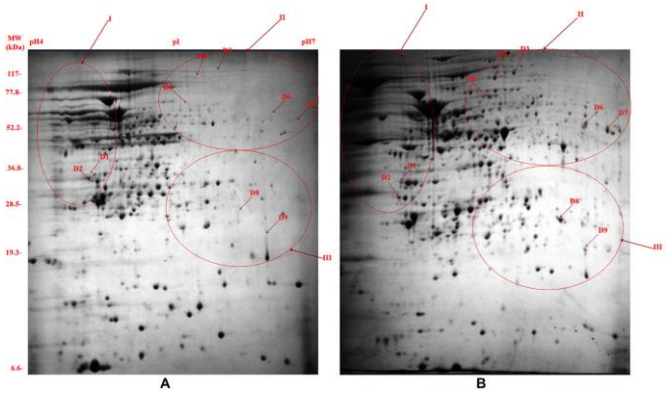

Figure 1: 2DE gels of lipophilic proteins of M. tuberculosis (marked spots were identified by MALDI-TOF/MS). (A) Extraction of lipophilic proteins directly by Triton X-114 phase separation. (B) Enrichment of lipophilic proteins by adding Triton X-100 (1% v/v) in sonication buffer followed by Triton X-114 phase separation.

Conclusion:

In conclusion, we have optimized an improved extraction protocol by adding Triton X-100 in the protein extraction buffer followed by Triton X-114 extraction (phase separation) directly without the need of preisolation of membranes. This protocol could be an efficient, rapid, and direct method for enriching the extraction of lipophilic proteins from M. tuberculosis H37Rv for 2DE.