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The protein-protein interaction network of *Mycobacterium tuberculosis* and novel antigenic protein discovery towards diagnostic and vaccine development

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Tuberculosis (TB) remains to be a major infectious disease throughout the world. However, the current vaccine for TB has variable protective efficacy, and there is no commercially available serodiagnostic test for this disease with acceptable sensitivity and specificity for routine laboratory use. We constructed a global protein-protein interaction (PPI) network for the human pathogen *Mycobacterium tuberculosis* H37Rv based on a high-throughput bacterial two-hybrid method. Almost the entire ORFeome was cloned and more than 8000 novel interactions were identified. The global network was linked to the protein secretion pathway. To identify novel antigenic candidates, we further screened systematically the novel antigenic proteins with the greatest potential as protective or diagnostic antigens by using the differential response of *M. tuberculosis* proteins to serum from TB patients and healthy individuals. Approximately 87% of the open reading frames of *M. tuberculosis* were successfully cloned and expressed in *Escherichia coli*, purified under denatured conditions, and tested for antigenicity using a mixture of sera from 15 TB patients. Out of the 3480 proteins screened, 249 proteins had significant reactions with the serum samples. Among the 249 proteins, 20 proteins were identified as most reactive. Compared with the commercial test kits, 3 novel antigens from the top 20 proteins, namely, Rv1987, Rv3807c, and Rv3887c, provided better sensitivity and accuracy. These newly identified antigenic proteins may be used as candidates for serodiagnostic application and vaccine development. Overall, our study's findings may serve as an essential reference for developing new TB diagnostic methods and more effective tuberculosis vaccines.

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