Utilization of *Bacillus subtilis* spores as mucosal vaccine delivery system for antigenic outer membrane proteins (OMPs) of *Acinetobacter baumannii*

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ABSTRACT

Introduction: The emergence of multidrug-resistant (MDR) *Acinetobacter baumannii* seriously threatens global public health and significantly contributes to healthcare costs. This bacterium has become a significant cause of nosocomial infections with limited treatment options. Therefore, alternative approaches are urgently needed to control the infections. Vaccines have shown the potential to reduce antibiotic usage, lessening the overall disease burden of infection and decreasing the spread of antimicrobial resistance. Developing a mucosal vaccine may be advantageous since *A. baumannii* infects various tissues; however, the development process is challenging, resulting in limited availability. **Objective:** This study aimed to establish *Bacillus subtilis* spore displaying antigenic outer membrane proteins (OMPs) of *A. baumannii* as a proof of concept for a subunit oral vaccine. **Materials and Method:** The identification of the antigenic protein within the OMPs was performed using reverse vaccinology. Four identified vaccine targets were utilized to construct recombinant DNA plasmids, propagated and transformed into *B. subtilis*. The recombinant *B. subtilis* was grown and induced into sporulation. The expression of the antigenic protein on the spore surface was detected using immunostaining assay and immunofluorescence microscopy. The spores expressing the OMPs were purified and inoculated into mice orally. The immune response was evaluated in the mice. **Results:** We identified 24 potential OMPs as vaccine targets and selected four OMPs in developing recombinant *B. subtilis*. The success in displaying the antigenic protein on the spore surface of recombinant *B. subtilis* were demonstrated. We assessed two out of four recombinant spore-displayed antigenic OMPs in mice, and the immune responses were evaluated. The findings showed that B. subtilis spore displaying TBDR proteins induced a humoral immune response against A. baumannii without causing toxicity in the mice. It also indicate the ability of the platform to sustain in the harsh physiochemical environment of the mucosa. **Conclusion:** *B. subtilis* spore-displayed OMPs should be further explored as potential mucosal vaccine candidates to prevent and control *A. baumannii* infection.