HIGH-THROUGHPUT SIGNATURE-TAGGED MUTAGENIC APPROACH TO IDENTIFY NOVEL VIRULENCE FACTORS OF YERSINIA PESTIS CO92 IN A MOUSE MODEL OF INFECTION

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Yersinia pestis (YP) is a Tier-1 select agent which causes bubonic, septicemic and/or pneumonic plague in humans. Pneumonic plague is a hyper-acute disease leaving only a short window for the treatment; however, currently there is no FDA approved vaccine against this disease. Therefore, identification of new virulence factors in YP and understanding their molecular mechanisms during an infection process are necessary in designing a better vaccine. Using a high-throughput, signature-tagged mutagenic approach, 5,088 mutants of YP CO92 were created and screened them in a mouse model of pneumonic plague at a dose equivalent to 5 LD50 of wild-type (WT) CO92. Of that, 118 clones showing impairment in disseminating to spleen were obtained. In the subsequent screen, 20/118 mutants exhibited attenuation at 8-40 LD50 when tested in a mouse model of bubonic plague. In-frame deletion mutation of two of the genes identified from the screen, namely rbsA that codes for a putative sugar transport system ATP-binding protein, and vasK, a component of the type-VI secretion system, were found to exhibit significant attenuation at 11-12 LD50 in a mouse model of pneumonic plague. Likewise, among the remaining 18 signature-tagged mutants, 9 were also attenuated at 12 LD50 in a pneumonic plague mouse model. Earlier, we reported that deletion of genes encoding Braun lipoprotein (Lpp) and acyltransferase (MsbB) reduced virulence of YP CO92 in mouse models of bubonic and pneumonic plague. Deletion of rbsA and vasK genes from either the Δlpp single or the ΔlppΔmsbB double mutant augmented the attenuation to provide 90-100% survivability to mice in a pneumonic plague model at 20-50 LD50s. The ΔlppΔmsbBΔrbsA triple mutant-infected mice at 50 LD50 were 90% protected upon subsequent challenge with 12 LD50 of WT CO92, suggesting that this mutant could potentially be further tested and developed into a live attenuated plague vaccine.

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