SEARCHING FOR NOVEL VIRULENCE FACTORS OF *YERSINIA PESTIS* BY HIGH-THROUGHPUT SIGNATURE-TAGGED MUTAGENIC APPROACH IN A MOUSE MODEL OF INFECTION

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*Yersinia pestis* (YP) is a Tier-1 select agent which causes plague in humans with three different pathodynamic manifestations, bubonic, septicemic and pneumonic. Pneumonic plague is a hyper-acute disease leaving only a short window for the treatment; usually 24 hr after contracting the agent. However, the most common form is the bubonic plague in humans which has a high mortality rate, if untreated. Therefore, prophylactic treatment with a vaccine is the best option; however, currently there is no FDA approved vaccine for human use against any form of plague. In order to design a better vaccine and formulate appropriate therapeutic interventions for the disease plague, identifying critical bacterial virulence factors and understanding their molecular mechanisms that are involved during the infection process are necessary. Therefore, we attempted to identify new virulence factors of YP strain CO92 in mouse model of bubonic/pneumonic plague using a high-throughput, signature-tagged mutagenic approach. In this regard, we created 5,088 mutants covering approximately one mutation per every 1000 bp of the genome using 53 unique DNA tags. Thus far, a total of 2,279 mutants have successfully been screened for their potential attenuation in a mouse model of plague, representing 44.79% of the total mutant clones created for this study. From this initial screening, 118 clones showing either complete or partial (101 and 17 clones, respectively) loss of virulence during the infection process were obtained via *in vitro* hybridization approach. Among 101 clones showing complete loss of virulence, 23 mutants were attenuated for developing bubonic plague in a mouse model, showing 60% or more survival rate even with the higher infectious doses of 25-50 LD$_{50}$ for some of these clones. Most of the attenuated mutants were identified as carrying mutational interruptions in uncharacterized genes encoding for hypothetical or putative proteins. Interestingly, one of the mutants was confirmed to carry disruption in the gene encoding a known virulence factor-plasminogen activator, adding credential to our screening process. When surviving mice were challenged with the wild type YP CO92 by the intranasal route to mimic pneumonic plague, 75-100% protection was observed, suggesting that these mutants could serve as better live attenuated vaccine candidates cable of elucidating strong immune protection.