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Isolation and characterization of ssDNA aptamers against BipD antigen of *Burkholderia pseudomallei*

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Abstract Background: Melioidosis is difficult to diagnose due to its wide range of clinical symptoms. The culture method is time-consuming and less sensitive, emphasizing the importance of rapid and accurate diagnostic tests for melioidosis. Burkholderia invasion protein D (BipD) of Burkholderia pseudomallei is a

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potential diagnostic biomarker. This study aimed to isolate and characterize single-stranded DNA aptamers that specifically target BipD. Methods: The recombinant BipD protein was produced, followed by isolation of BipD-specific aptamers using Systematic Evolution of Ligands by EXponential enrichment. The binding affinity and specificity of the selected aptamers were evaluated using Enzyme-Linked Oligonucleotide Assay. Results: The fifth SELEX cycle showed a notable enrichment of recombinant BipD protein-specific aptamers. Sequencing analysis identified two clusters with a total of seventeen distinct aptamers. AptBipD1, AptBipD13, and AptBipD50 were chosen based on their frequency. Among them, AptBipD1 exhibited the highest binding affinity with a K_d value of 1.0 μ M for the recombinant BipD protein. Furthermore, AptBipD1 showed significant specificity for *B. pseudomallei* compared to other tested bacteria. Conclusion: AptBipD1 is a promising candidate for further development of reliable, affordable, and efficient point-of-care diagnostic tests for melioidosis.

Keywords

Author Keywords: Melioidosis; *B. pseudomallei*; BipD; ssDNA aptamers; SELEX; ssDNA aptamers; SELEX

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