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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 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 Kd value of 1.0 μM 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. © 2024

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

Index Keywords
Antigens, Oligonucleotides; Aptamers, B pseudomalleus, Binding affinities, Burkholderia, Burkholderia invasion protein D, Burkholderia pseudomallei, Diagnostic tests, Melioidosis, SELEX, SsDNA aptamer; Binding energy; aptamer, aptbipd1, aptbipd13, aptbipd50, bacterial antigen, biological marker, Burkholderia invasion protein D, double stranded DNA, n hydroxysuccinimide, recombinant protein, single stranded DNA, unclassified drug, aptamer, bacterial antigen, bacterial protein, recombinant protein, single stranded DNA; antigen detection, Article, bacterium detection, binding affinity, Burkholderia cepacia, Burkholderia pseudomallei, Burkholderia thailandensis, cloning vector, diagnostic accuracy, DNA library, DNA synthesis, enzyme linked oligonucleotide assay, Escherichia coli, immunoassay, in vitro selection, melioidosis, molecular cloning, nonhuman, nucleotide sequence, phylogeny, point of care testing, polymerase chain reaction, protein expression, protein isolation, protein purification, protein secondary structure, recombinant plasmid, Salmonella enterica serovar Paratyphi A, Salmonella enterica serovar Paratyphi B, Salmonella enterica serovar Paratyphi C, Salmonella enterica serovar Typhi, Salmonella enterica serovar Typhimurium, Sanger sequencing, sequence analysis, Shigella boydii, Shigella flexneri, Shigella sonnei, single strand break repair, systematic evolution of ligands by exponential enrichment aptamer technique, type III secretion system, chemistry, diagnosis, genetics, human, isolation and purification, melioidosis, microbiology, systematic evolution of ligands by exponential enrichment aptamer technique; Antigens, Bacterial, Aptamers, Nucleotide, Bacterial Proteins, Burkholderia pseudomallei, DNA, Single-Stranded, Humans, Melioidosis, Recombinant Proteins, SELEX Aptamer Technique

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