World Academy of Science, Engineering and Technology
International Journal of Biotechnology and Bioengineering
Vol:16, No:07, 2022

Design of DNA Origami Structures Using LAMP Products as a Combined System for the Detection of Extended Spectrum B-Lactamases

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Abstract : The group B-lactamic antibiotics include some of the most frequently used small drug molecules against bacterial infections. Nevertheless, an alarming decrease in their efficacy has been reported due to the emergence of antibiotic-resistant bacteria. Infections caused by bacteria expressing extended Spectrum B-lactamases (ESBLs) are difficult to treat and account for higher morbidity and mortality rates, delayed recovery, and high economic burden. According to the Global Report on Antimicrobial Resistance Surveillance, it is estimated that mortality due to resistant bacteria will ascend to 10 million cases per year worldwide. These facts highlight the importance of developing low-cost and readily accessible detection methods of drug-resistant ESBLs bacteria to prevent their spread and promote accurate and fast diagnosis. Bacterial detection is commonly done using molecular diagnostic techniques, where PCR stands out for its high performance. However, this technique requires specialized equipment not available everywhere, is time-consuming, and has a high cost. Loop-Mediated Isothermal Amplification (LAMP) is an alternative technique that works at a constant temperature, significantly decreasing the equipment cost. It yields double-stranded DNA of several lengths with repetitions of the target DNA sequence as a product. Although positive and negative results from LAMP can be discriminated by colorimetry, fluorescence, and turbidity, there is still a large room for improvement in the point-of-care implementation. DNA origami is a technique that allows the formation of 3D nanometric structures by folding a large single-stranded DNA (scaffold) into a determined shape with the help of short DNA sequences (staples), which hybridize with the scaffold. This research aimed to generate DNA origami structures using LAMP products as scaffolds to improve the sensitivity to detect ESBLs in point-of-care diagnosis. For this study, the coding sequence of the CTM-X-15 ESBL of E. coli was used to generate the LAMP products. The set of LAMP primers were designed using PrimerExplorerV5. As a result, a target sequence of 200 nucleotides from CTM-X-15 ESBL was obtained. Afterward, eight different DNA origami structures were designed using the target sequence in the SDCadnano and analyzed with CanDo to evaluate the stability of the 3D structures. The designs were constructed minimizing the total number of staples to reduce costs and complexity for point-of-care applications. After analyzing the DNA origami designs, two structures were selected. The first one was a zig-zag flat structure, while the second one was a wall-like shape. Given the sequence repetitions in the scaffold sequence, both were able to be assembled with only 6 different staples each one, ranging between 18 to 80 nucleotides. Simulations of both structures were performed using scaffolds of different sizes yielding stable structures in all the cases. The generation of the LAMP products were tested by colorimetry and electrophoresis. The formation of the DNA structures was analyzed using electrophoresis and colorimetry. The modeling of novel detection methods through bioinformatics tools allows reliable control and prediction of results. To our knowledge, this is the first study that uses LAMP products and DNA-origami in combination to delect ESBL-producing bacterial strains, which represent a promising methodology for diagnosis in the point-of-care.

Keywords : beta-lactamases, antibiotic resistance, DNA origami, isothermal amplification, LAMP technique, molecular diagnosis
Conference Title : ICBN 2022 : International Conference on Biotechnology and Nanotechnology
Conference Location : Tokyo, Japan
Conference Dates : July 21-22, 2022