„Production, characterization and application of fusion forms of archeal DNA polymerase”

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The rapid advancement of biotechnological techniques, including PCR-based methods, requires a search for new and improved enzymes for nucleic acid amplification. This method serves as the foundation for molecular diagnostics of infectious and genetic diseases. It is also widely used in food and beverage, veterinary, and forensic industries. PCR inhibitors and matrices rich in G-C pairs pose numerous challenges to such a widely used technique. The coronavirus pandemic has revealed technological issues with the high-throughput enzyme production as well as the quality of some commercially available products. Despite the large number of available enzymes, the new molecules are still required to advance diagnostics.

In the last 20 years, many new DNA polymerases, as well as their improved versions, have appeared on the market. A breakthrough in the design of high-performance polymerases was the use of small nucleic acid binding proteins as DNA strand stabilizing proteins during amplification. The future of high-yield commercial polymerases lies in the application of genetic bioengineering to design fusion forms of archaeal DNA polymerases.

The aim of this doctoral thesis was to obtain and characterize three fusion variants of archaeal DNA polymerase (V1 Pfu-Sso7d-Sso7d, V2 Sso7d-Pfu-Sso7d and V3 NeqSSB-Pfu-Sso7d). In the course of the work, the influence of two DNA-binding proteins on the properties of DNA polymerases was determined. Modifications of Pfu polymerase were investigated due to its lowest percentage of errors and high thermal stability.

Each thermostable DNA polymerase has its own set of unique characteristics, including thermostability, DNA elongation rate, fidelity, processivity, specificity, resistance to contaminants and inhibitors. The distinctive properties of each DNA polymerase can be exploited to create unique variants of DNA polymerases. Many studies indicate that even small differences in the sequence of amino acids and the addition of a DNA binding protein can result in huge changes in their biochemical properties. Thus, it suggests that it is possible to obtain new DNA polymerases with improved functionality.

All properties of the obtained archaeal DNA polymerase fusion variants were compared to the popular Pfu polymerase modification, Ref1 Pfu-Sso7d, which was purified in parallel with the other variants and used as a reference. The obtained results allowed for the selection of the most promising variant - V2 Sso7d-Pfu-Sso7d. It stood out from the other three due to its unique properties, which have not been reported in the literature yet. The V2 Sso7d-Pfu-Sso7d polymerase obtained through modification enabled a development of an

enzyme with a wide tolerance to changing reaction conditions and extreme resistance to inhibitors. The presence of two identical DNA-binding proteins such as Sso7d, placed at both ends of DNA polymerase V2 Sso7d-Pfu-Sso7d, allows to carry out PCR under different reaction conditions, i.e. with a high tolerance to salt concentrations and a wide range of tolerance to the applied pH; with exceptional activity, allowing for efficient DNA amplification in extremely difficult reaction conditions; with high resistance to inhibitors found in clinical samples, such as blood, heparin, ampicillin or humic acid; preservation of other valuable properties of the reference DNA polymerase, such as thermostability, resistance to some inhibitors, the ability to amplify the so-called difficult matrices rich in GC pairs, with different kinetics of interaction with nucleic acids and with increased sensitivity in relation to the reference DNA polymerase.

In addition, an archaeal DNA polymerase fusion form containing the NeqSSB protein from *Nanoarcheum equitans* was studied. In comparison to the reference polymerase Ref1 Pfu-Sso7d, the obtained polymerase demonstrated greater sensitivity, higher activity, and resistance to inhibitor such as whole blood. The obtained results may contribute to advancement of broadly understood diagnostics.

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