IDENTIFICATION AND QUALITATIVE EXPRESSION ANALYSIS OF UNUSUAL ESTERASE FROM *STAPHYLOCOCCUS SAPROPHYTICUS* AG1

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It is well-known that bacterial lipolytic enzymes are extremely attractive for the sustainable industry applications due to their unique biocatalytic properties. These enzymes are valued not only for their natural ability to catalyze hydrolysis of carboxylic esters, but also for the *in vitro* promiscuity and ability to catalyze carboxylic ester bond synthesis and other non-specific activities. Easy extraction of bacterial lipolytic enzymes, their stability in various reaction conditions (extreme temperatures, pH, and chemical agents), wide range of substrates enable their applications in the fields of oleochemistry, polymer, textile, detergent, food, cosmetic industries, biodiesel production and bioremediation. With one of the most exceptional properties of enantioselectivity, bacterial lipolytic enzymes are distinguished as one of the most important biocatalysts used to produce optically pure chemical compounds [1, 2]. Their biotechnological significance is also reflected by the fact that certain microbial lipases and esterases are commercialized by the global biotechnology companies such as Fluka, Novozymes, Biocatalysts Ltd and other [3, 4]. Nevertheless, the demand for these biotechnologically relevant enzymes is not diminishing. Also, despite the current high number of identified lipolytic enzymes (~ 5000), only a small percentage (<10 %) of them are characterized [5]. Moreover, physiological relevance of these enzymes in bacteria are also underestimated and not fully understood and studied.

With the significant improvement of genome sequencing over the past decades, large amounts of data have become available in the public databases, analysis of which allows identification of new enzymes and helps to fill in the gap of knowledge regarding certain enzymes and other biological molecules. Moreover, genome-mining approach allows easy discovery of new biocatalysts more purposefully and without some expenses needed by utilizing classical microbiological enrichment culture approach or metagenomic analysis and protein engineering [6].

In this work, unusual EstAG1 esterase with unconventional conservative amino acid motives was identified to be coded in the genome of *Staphylococcus saprophyticus* AG1. Because of the unusual sequence traits of the EstAG1 enzyme, in order to preliminary evaluate its possible function and nature of the expression of the gene in the native bacterial cells, qualitative expression analysis was performed. For that purpose, bacterial cells of *S. saprophyticus* AG1 were grown in different nutrient composition media. For the amplification of EstAG1, total RNA was extracted from different growth phases of the bacteria and one-step RT-PCR method was employed.

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