doi 10.18699/bgrs2024-8.2-14

Differential expression of two alkane oxidation systems in response to hydrocarbon exposure in *Tsukamurella tyrosinosolvens* PS2

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Key words: alkane; *alkB*; *P450*; alkane-degradation

Motivation and Aim: Microbial bioremediation is considered one of the most effective and cost-efficient approaches to remove hydrocarbons from contaminated ecosystems. The transformation and mineralization of organic pollutants by these microorganisms occurs due to the ability to use toxic substances to meet their energy and carbon needs. Most alkane-oxidizing bacteria contain a hydroxylase system, which is encoded by the *alkB* gene cluster. In addition, it has been shown that cytochrome *P450* is involved in the oxidation of alkanes with a chain length of C5-C16. There is some published data that alkane-oxidizing bacteria contain both *alkB* and *P450* systems [1], but it is not clear how they interact at the regulatory level and which one will play a dominant role in alkane degradation. The *T. tyrosinosolvens* PS2 strain is capable of using alkanes with different chain lengths as the sole carbon and energy source [2]. The present study aimed to evaluate the expression of alkane hydroxylase genes, such as *alkB* and *P450*, induced by alkanes of different chain lengths to elucidate the high biodegradability of PS2 strain.
Methods and Algorithms: Total RNA from PS2 strain cells was isolated with Qiagen RNeasy Mini kit according to the manufacturer's instruction with some modifications, under different cultivation conditions (sucrose, dodecane, hexadecane, hexatriacontane, liquid paraffin), cDNA was obtained, and real-time PCR was performed with specific primers to evaluate gene expression.
Results: Real-time PCR analysis showed that alkanes with different chain lengths can affect the two studied genes *(alkB* and *P450*), which leads to differences in expression between these genes when PS2 cells are grown under different culture conditions. Interestingly, on a medium supplemented with dodecane, the expression of the *P450* gene increased by 2,851 times, and the *alkB* gene by 1,229 times. The increase in P450 gene expression was also predominant in the medium supplemented with liquid paraffin and hexadecane as the sole energy source, compared to the *alkB* gene. On the other hand, on a medium supplemented with hexatriacontane, the expression of the *alkB* gene was increased by 32 times, and the *P450* gene by 7 times. Thus, a switch from the *P450* gene to the *alkB* gene is observed depending on the length of the hydrocarbon chain.
Conclusion: The obtained results demonstrated a preferential activation of the *P450* gene on medium-chain alkanes, although most studies describe, on the contrary, an increase in the expression of the *alkB* gene. The regulatory mechanisms identified in this study will be used to create tools to control cellular processes, increasing metabolic rates and the efficiency of biocatalysis.

Funding: This research was funded by the subsidy allocated to the Kazan Federal University for the state research assignment (No. FZSM-2023-0013) and the Kazan Federal University Strategic Academic Leadership Program (PRIORITY-2030).

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