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Enhancing the performance of *Methylorubrum extorquens* AAZ-1 by using CRISPR/Cas9 for the degradation of hydrocarbon compounds

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Abstract. An efficient expression method was used to create a genetic modification strategy for methylotrophic bacteria to improve the capacity of bioremediating bacteria to absorb oil in contaminated environments. Using the plasmid-treated CRISPR-Cas9 system, *Methylorubrum extorquens* AAZ-1(OR226417.1) was treated, and qPCR analysis revealed that the expression of the *MxaF* gene (encoding methanol dehydrogenase enzyme) rose six-fold. To increase the biodegradation effectiveness of hydrocarbons (n-alkane and PAH), CRISPR-Cas9 technology was selected for the investigation and use in the laboratory. The biodegradation rate of n-alkane in the modified *M. extorquens* AAZ-1 rose from 61.14% to 74.35% during a seven-day incubation period when compared to the control. The proportion of polycyclic aromatic hydrocarbons in the altered *M. extorquens* AAZ-1 rose from 65.69% to 78.23%. To the best of the authors' knowledge, this work is the first time that a CRISPR-Cas9 system has been employed to improve the efficiency of these bacteria in the biodegradation of hydrocarbon molecules.

Keywords: biodegradation, CRISPR-Cas9, methanol dehydrogenase, *Methylorubrum extorquens* AAZ-1

INTRODUCTION

A serious global environmental problem is the pollution of soil by crude oil and its compounds. Many hydrocarbon compounds have the potential to change the natural soil or water conditions. They can permeate ecosystems and build up in both plant and animal tissues, resulting in a variety of harmful effects, including cancer induction, mutations, as well as respiratory and brain system problems (Asemoloye *et al.*, 2020; Naveed *et al.*, 2020). Microorganisms may use hydrocarbons as an energy source by decomposing them, so their use in bioremediation procedures is a logical outcome (Ławniczak *et al.*, 2020). Methylotrophs are microorganisms that can produce biomass from single-carbon substances like methane, methanol, or formate, as well as multicarbon substances that lack carbon-carbon bonds. Methylotrophic bacteria have enzymes that seem suitable to employ in the processes of bioremediation and biodegradation due to their ability to oxidize CH_3OH to HCHO and $HCOO^-$, as well as their non-formation of unwanted products (Klein *et al.*, 2022; Qiu *et al.*, 2014).

Bioremediation, also known as self-cleaning, relies on bacterial populations already present in contaminated soil to remove oil without the use of any specific technology. Oil bioremediation is acknowledged to be a practical and eco-friendly method on a global scale (Ejaz *et al.*, 2021). The gold standard for researchers studying the genetic underpinnings of physiological and metabolic processes of any organism, particularly the important bacteria used in science and industry, is genome editing (Arroyo-Olarte *et al.*, 2021). The gene-editing approach supplies the best microbe with complex genes, resulting in a more distinct microorganism (Dangi *et al.*, 2019; Basu *et al.*, 2018). As a result, it is possible to obtain changed genetic sequences of distinct wild types (Dai *et al.*, 2018; Stein *et al.*, 2018).

The gene editing procedure begins with the creation of a complementary guide sequence to

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