Mechanistic Insights into Benzo[a]pyrene Degradation by Halotolerant Mn-Oxidizing Bacteria and Biogenic Mn Oxides in Soil

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Abstract

The problem of petroleum pollution in soil has raised significant concerns, with polycyclic aromatic hydrocarbons (PAHs) being typical pollutants in oil-contaminated soil. Salt-alkaline soil, in particular, exacerbates the PAH pollution issue due to nutrient deficiencies, low microbial abundance, and salinization. Microbial remediation technology is currently a promising in-situ remediation technique for contaminated soil, with broad application prospects. Manganese-oxidizing bacteria (MnOB) are a type of microorganisms that can form biogenic manganese oxides (BMOs). BMO is a type of mineral with oxidation ability second only to O_2 and the most reactive. BMO produced by MnOB, along with the highly oxidative intermediates released (superoxide, Mn(III), H_2O_2, etc.), exhibit strong oxidation and adsorption capabilities, efficiently removing various pollutants such as arsenic, endocrine disruptors, antibiotics, pesticides, etc. However, there is relatively little research on the performance of MnOB in degrading PAHs in saline-alkali soil. Consequently, this study isolated and purified two salt-tolerant MnOB strains, *Halobacillus marinus* strain YJT-1 and *Bacillus cereus* strain TX-1, through enrichment culture and gradient dilution. Analyzed their Mn(II) oxidation ability under different salinities, varying Mn(II) concentrations, and different

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carbon sources. Opted for saline-alkali soils from Xinjiang, Ningxia, and Shandong as the research specimens. Study the degradation capability of salt-tolerant MnOB towards Benzo[a]pyrene (BaP). Identified the primary constraints on BaP degradation by salt-tolerant MnOB through the application of statistical techniques like principal component analysis and multiple linear regression analysis. Elucidated the functional genes and microbial community changes in MnOB degradation of PAHs through quantitative Q-PCR, high-throughput sequencing, and metagenomics techniques. The results showed that YJT-1 exhibited the highest biological manganese oxidation (BMO) yield (97.83 mg/L) at 100 mg/L Mn(II), 3.5% salinity, and L-glutamic acid as the carbon source, while TX-1 reached 85.21 mg/L under similar conditions. During soil remediation trials, YJT-1 and TX-1 both significantly facilitated the BaP in Xinjiang soil (39% on day 7) and Ningxia soil (53% on day 14) respectively, surpassing the control and the model strain, *Pseudomonas putida* MnB1. Analysis of microbial communities indicated that YJT-1 and TX-1 showed enrichment of functional genera (*Stutzerimonas, Aromatoleum, Pseudomonas*) and elevated levels of crucial metabolic pathways (naphthalene/benzoate degradation, oxidative phosphorylation). Functional genes confirmed the involvement of *phe*, *pcaGH*, and *benAB-xylY* in PAH degradation, as well as manganese transport (*sit, mnt*) and Na^+ transport (*maeN, oadA*) genes. Salt-tolerant MnOB can degrade PAHs pollution in saline-alkali soil through mechanisms such as reactive oxygen species ($\cdot O_2^-$, H_2O_2, $\cdot OH$) and enzyme-catalyzed oxidation (multicopper oxidase, peroxidase).This study provides a feasible strategy for using salt-tolerant MnOB in PAH-contaminated soil with salinity.

Keywords: Bioremediation; Polycyclic aromatic hydrocarbons (PAHs); Benzo[a]pyrene (BaP); Mn(II)-oxidizing bacteria (MnOB); Salt-alkaline soil