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EVALUATION OF THE EFFICIENCY OF A BACTERIAL CONSORTIUM IN DEGRADING POLYCYCLIC AROMATIC HYDROCARBONS (PAH) UNDER THE INFLUENCE OF AND WITHOUT COPPER (CU) METAL STRESS

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Polycyclic aromatic hydrocarbons (PAHs) are persistent organic pollutants found extensively in petroleum-contaminated environments. They pose a severe risk due to their carcinogenic, mutagenic, and bio-accumulative properties. This study investigates the ability of a certain selected bacterial consortium to degrade PAHs, specifically naphthalene and phenanthrene, under nutrient-limited conditions and copper (Cu) stress as the heavy metal. Primary bacterial isolates obtained from environmental samples were screened on Bacto Bushnell-Haas agar infused with PAHs as the sole carbon source for primary screening. Effective colonies were then subjected to secondary screening using methylene blue as a redox indicator, and the absorbance was quantified through a spectrophotometric analysis at the absorbance level of 609nm. These methods include culturing bacterial colonies in Bushnell-Haas broth, with varying concentrations of PAHs and copper sulphate (20 ppm, 40 ppm, 60 ppm, 80 ppm, and 100 ppm). Absorbance reading indicated effective PAH degradation, particularly at 20 ppm, 40 ppm, and 80 ppm for phenanthrene and 20 ppm and 60 ppm for naphthalene. Triplicate experiments ensured statistical reliability. Initial confirmation of bacterial strains M11, C1, G13, M20, and J6 was carried out using Gram staining. The confirmed isolates were then subjected to antagonistic assays, which verified their mutual compatibility and diversity for effective consortium-based PAH degradation. Bacterial strains survived nutrient starvation and utilized naphthalene and phenanthrene as sole carbon sources. Primary screening identified efficient colonies, which were further evaluated through secondary screening using absorbance-based measurements. After 7 days, a phenanthrene-treated sample without copper showed a decrease in absorbance from 0.295 to 0.122, indicating 69.13% degradation. Under copper-induced stress, the consortium remained metabolically active, though with slightly reduced degradation.The isolates retained metabolic activity even under copper-induced stress, specifically at a concentration of 81.32 mg/kg of Cu, confirming their tolerance and resilience in heavy metal-contaminated environments, making them strong strains for an eco-friendly environment, contributing to advancing microbial bioremediation technologies and providing insights into PAH biodegradation under dual-stress conditions. This research highlights the potential applications of bacterial consortia in bioremediation targeting PAH contamination in metal-stressed environments.

Keywords: bioremediations, bacteria consortium, copper stress, naphthalene, phenanthrene

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Introduction

Polycyclic aromatic hydrocarbons, also known as PAHs, are toxic, carcinogenic, and harmful pollutants composed of two or more fused rings, primarily originating from petroleum contamination, incomplete combustion of organic matter, and industrial waste. PAHs persist in the environment and pose a significant risk to human health and the ecosystem due to their bioaccumulative and mutagenic properties. Traditional methods of bioremediation have proven to be cost-intensive and damaging to the environment. Microbial bioremediation offers promising yet eco-friendly and cost-effective alternatives, increasing the natural degradation ability of certain bacterial consortia.

In recent years, bacterial consortia have emerged as a superior strain for bioremediation as its more effective compared to a single strain. It's their metabolic diversity and synergetic activities that enable them to degrade a wider range of PAHs more efficiently. However, environmental factors such as nutrient availability and heavy metal stress (copper) can influence the capacity for degradation. This study investigates the degradation efficiency of a laboratory-developed bacterial consortium under conditions of nutrient limitation and increasing copper concentrations.

Bioremediations, particularly using microbial approaches, have been gaining significant attention due to its potential in degrading and removing harmful contaminants such as PAHs. PAHs are classified into low and high-molecular-weight compounds. Low molecular weight PAHs, such as naphthalene, are more soluble and degradable. While high molecular weight compounds such as benzo[a]pyrene are more persistent and toxic in breaking these bonds. The ubiquity of PAHs in industrial sites, vehicular emissions, and oil spills leads to innovative remediation strategies that are both effective and environmentally sustainable.

Even though copper is essential in trace amounts, having a high concentration level above the hazardous level can be dangerous to the environment as well as humans. It interferes with microbial enzymatic activities, if bacteria and cell membranes are stable. Therefore, looking into microbial consortia under these conditions, such as dual stress conditions, nutrient limitations, and metal toxicity, shows how well the bacterial consortia are applicable in polluted environments. The objective of this research is not only to evaluate the degradation efficiency but also to analyze the mechanism of microbial communities in response to metal stress.

Methodology

1. Bacterial isolation and starvation screening

Bacterial strains were isolated using soil samples enriched with Bushnell-Haas broth, a nutrient-deficient medium designed to promote PAH degradation. Bacterial starvation was done on BBH agar plates inoculated with the isolated colonies taken from streak plates. This medium lacks a carbon source, making the bacteria rely only on PAHs such as naphthalene and phenanthrene as their sole energy source.

2. Primary screening for PAH degradation

Colonies that showed survival under starved conditions were then subjected to primary screening again on BBH agar with 1mg/ml of both naphthalene and phenanthrene, which was dissolved with acetone before screening. The plates were yet again incubated at 28°C. The colonies that were able to degrade were selected for secondary screening.

3. Secondary screening using methylene blue

The decolorization of methylene blue was used as an indicator of microbial respiration and PAH degradation. Test tubes containing PAHs, bacterial inoculum, and methylene blue were prepared in triplicate for better reliability. Absorbance was measured at 609nm using a spectrophotometer after 8 days of incubation. Decreased absorbance indicated that there was increased microbial activity and degradation.

4. Effect of copper stress

To check for environmental stress, the BBH broth was mixed with copper sulphate, and both the PAHs were suspended together, keeping one control and one sample. Except that the control didn't have the bacteria, and the sample had a bacterial consortium. The copper sulphate concentration ranged from 20-100 ppm, as the copper concentration level was 81%. The degradation percentage was calculated using the following formula:

Degradation (%) = ((C1 - C2) / C1) × 100

Where:
C1 = Absorbance of control
C2 = Absorbance of sample

Results & Discussion

Bacterial strains survived starvation and showed growth on naphthalene and phenanthrene as their sole carbon source. Primary screening identified the most efficient colonies for further studies in this research. In secondary screening, the absorbance reading showed significant colour loss in tubes showing bacterial degradation. For example, absorbance dropped from 0.295 to 0.122(69.13% degradation) in one sample. In copper stress, the consortium remained active at certain concentrations.

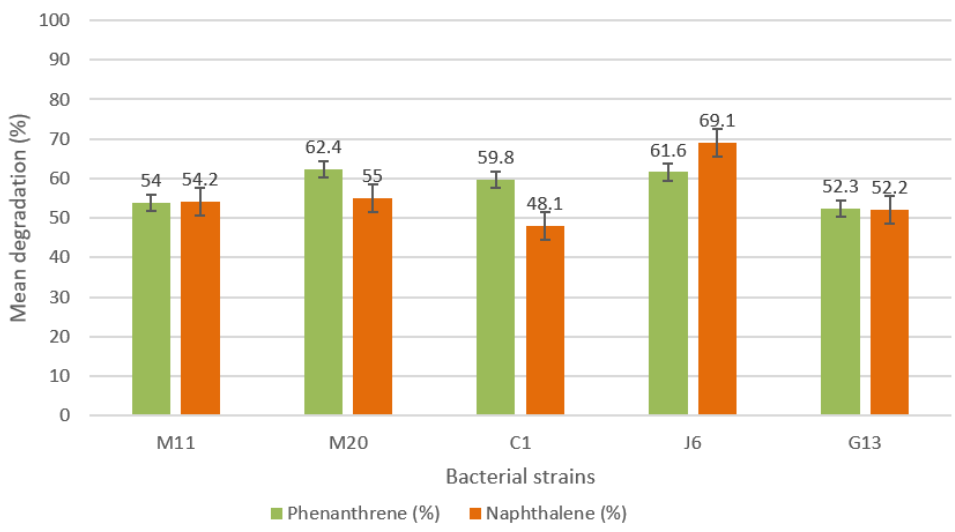

Figure 1: PAH degradation under secondary screening conditions.

The study confirms the potential it has for biodegradation of a mixed bacterial consortium against PAHs, particularly under nutrient-limited conditions, using BBH media. The starvation process effectively selected PAH-degradation strains. Methylene blue acted as a visual and analytical indicator of degradation. The copper stress experiment provided critical insights into the impact of heavy metals on microbial degradation. while low to moderate concentrations of $CuSO4$ (20–60 ppm) showed bacterial activity, higher concentrations (100 ppm) likely inhibited these enzymatic pathways, which are essential for degradation.

The result showed that bacteria can break down PAHs better when metal levels (copper) are low or not too toxic. As the metal concentration gets higher, it becomes harder to degrade, changing the function of bacteria and their function and unable to degrade properly. To make the test more reliable and efficient, the test was repeated thrice as a triplicate to ensure the value and consistency. In comparison to the control, which includes copper and PAHs with and without the bacteria as to make the findings stronger.

Studies show that bacterial consortia consist of genera such as Pseudomonas, Bacillus, which show significant tolerance to metal stress, maintaining their function to degrade. Yet the consortium developed in this study needs further study on detailed information about the bacteria. Further DNA sequencing and enzymatic assay could be done to have detailed information about what kind of bacteria our study shows.

Conclusion & recommendations

This study demonstrates the ability of an isolated bacterial consortium to degrade naphthalene and phenanthrene under nutrient-limited conditions with copper-induced stress. This highlights the potential application of bacterial consortia in real-world bioremediation, especially in contaminated environments. This research contributes to optimizing bioremediation on the active strains involved.However, further enhancement of remediation efficiency can be achieved through advanced techniques. Future research should explore genetically engineered bacteria (GEMs) for targeted degradation, integrate bioaugmentation and biostimulation strategies to strengthen microbial activity, and investigate phytoremediation and enzyme-based bioremediation approaches for synergistic pollutant removal. These advancements could significantly improve the scope and applicability of bioremediation in complex contaminated environments.

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