MULTIFUNCTIONAL SOIL BACTERIA: NAPHTHALENE DEGRADATION AND PLANT GROWTH PROMOTION IN VARYING SOIL CONDITIONS

[1]Komal Rani, [2]Dr. Dharma Dev Pandey
[1]Research Scholar, [2]Supervisor
[1-2] Department of Botany, Malwanchal University, Indore, Madhya Pradesh, India

Abstract
Soil contamination by pollutants such as naphthalene poses significant challenges to agricultural productivity and environmental health. Multifunctional soil bacteria that simultaneously degrade naphthalene and promote plant growth present a sustainable solution for bioremediation. This paper explores the dual role of these bacteria, focusing on their capacity for naphthalene degradation and their contribution to plant health. We review the mechanisms of microbial adaptation to polluted soils, the isolation and screening of efficient bacterial strains, and the influence of bacteria on soil remediation and plant growth promotion. Laboratory and field experiments are analyzed to evaluate the efficacy of bacterial inoculation in improving plant resilience and soil fertility, even under contaminated conditions. The potential of these bacteria to enhance bioremediation efforts and support sustainable agriculture highlights their significance in maintaining soil health in polluted environments.
Keywords: Naphthalene degradation, plant growth promotion, bioremediation, soil bacteria, microbial adaptation, soil contamination, PGPR

INTRODUCTION

Contextualizing the Dual Role of Bacteria in Bioremediation and Agriculture

Soil contamination with toxic hydrocarbons, such as naphthalene, is a pressing environmental issue, impacting ecosystems and agriculture alike. Among the various solutions to address soil pollution, the use of soil bacteria has emerged as an effective and sustainable approach. Certain bacteria possess the ability to degrade hydrocarbons, thereby detoxifying the soil—a process known as bioremediation. Concurrently, many of these same bacteria also play a supportive role in promoting plant growth. Known as plant growth-promoting rhizobacteria (PGPR), these bacteria offer benefits to plants by enhancing nutrient availability, producing growth hormones, and improving stress tolerance. This dual functionality, where bacteria simultaneously clean polluted environments and support agricultural productivity, exemplifies the unique adaptability and value of these microorganisms. By focusing on bacteria capable of both bioremediation and plant growth promotion, we can create a synergistic solution that restores soil health and boosts plant resilience.

Significance of Naphthalene Degradation in Polluted Soils

Naphthalene, a polycyclic aromatic hydrocarbon (PAH), is a common contaminant in soils near industrial zones, transportation hubs, and areas exposed to fossil fuel use and spills. Classified as an environmental pollutant, naphthalene poses risks to soil quality and ecosystem health due to its toxicity and persistence. In soils, naphthalene can hinder microbial diversity and impair soil fertility by creating an environment hostile to many beneficial organisms. Furthermore, naphthalene can disrupt plant growth, causing inhibited root development and reducing overall productivity. Thus, the degradation of naphthalene is essential not only for detoxifying contaminated soils but also for restoring an environment conducive to plant growth. Bacteria that can effectively degrade naphthalene break it down into less toxic intermediates, ultimately transforming it into harmless compounds, such as carbon dioxide and water. This microbial action not only remediates polluted soils but also paves the way for agricultural practices in areas previously considered unsuitable for cultivation.

Exploration of the Plant Growth-Promoting Potential of These Bacteria

Beyond their bioremediation abilities, many naphthalene-degrading bacteria are also recognized for their potential to promote plant growth. These bacteria contribute to plant health through various mechanisms, including nitrogen fixation, phosphate solubilization, and the production of plant growth hormones, such as auxins and cytokinins. Nitrogen fixation, for instance, is critical in providing plants with usable forms of nitrogen, which is essential for growth but often limited in availability in contaminated soils. Phosphate solubilization increases phosphorus availability, further supporting plant nutrition. Additionally, the production of phytohormones by these bacteria can stimulate root elongation and improve nutrient uptake, which is especially valuable in stressed or polluted environments. By promoting plant health, these multifunctional bacteria enhance the resilience and productivity of plants, making them vital allies in both sustainable agriculture and environmental restoration.

SOIL CONTAMINATION AND MICROBIAL ADAPTATION

Impact of Naphthalene on Soil Health and Plant Growth

Naphthalene contamination in soils has far-reaching consequences for soil health, microbial diversity, and plant development. This toxic hydrocarbon disrupts the natural balance of the soil ecosystem, often leading to a reduction in the population of beneficial microbes that play crucial roles in nutrient cycling and soil structure. In addition to degrading soil health, naphthalene is known to inhibit plant growth by interfering with root development, nutrient absorption, and overall plant vigor. Plants in contaminated soils may exhibit stunted growth, chlorosis, and a reduced ability to resist environmental stresses, making naphthalene a significant barrier to successful plant cultivation. For agriculture, this pollution translates to lower crop yields and compromised soil productivity, which highlights the urgent need for effective soil remediation strategies to mitigate the adverse effects of naphthalene contamination.

Microbial Adaptation Mechanisms in Contaminated Environments

Microorganisms in contaminated soils often develop specialized adaptations that allow them to survive and function despite the presence of toxic compounds like naphthalene. One of the primary adaptation mechanisms is the evolution of metabolic pathways capable of degrading hydrocarbons. For instance, bacteria that can metabolize naphthalene possess enzymes such as naphthalene dioxygenase, which initiates the breakdown of naphthalene by converting it into less toxic intermediates. This adaptation allows the bacteria to use naphthalene as a carbon source, facilitating their survival in polluted soils. Additionally, some bacteria form biofilms—a protective layer that allows microbial communities to resist the toxic effects of contaminants while supporting the breakdown of hydrocarbons. Through these adaptive strategies, microbial communities not only survive in contaminated environments but also contribute to soil health by removing pollutants. Such adaptations underscore the resilience of soil bacteria and their potential to be harnessed for effective bioremediation.

The Synergistic Relationship Between Soil Bacteria and Plants

The interaction between soil bacteria and plants is mutually beneficial, especially in contaminated soils where plants rely on microbial assistance to mitigate the effects of pollution. Bacteria that degrade naphthalene and promote plant growth help plants establish and thrive in soils that would otherwise be too toxic for cultivation. Plants, in turn, secrete root exudates that supply bacteria with essential nutrients, creating a favorable environment for microbial activity. This synergistic relationship enables plants and bacteria to support each other's survival and growth. For example, plants in naphthalene-contaminated soils benefit from bacteria that break down the pollutant, reducing its toxicity in the rhizosphere. Simultaneously, these bacteria provide plants with enhanced access to nutrients and growth-stimulating compounds, which aid in root development, nutrient uptake, and stress tolerance. This partnership not only facilitates plant growth in challenging environments but also contributes to the restoration of soil health, underscoring the importance of using multifunctional bacteria in both bioremediation and sustainable agriculture.

ISOLATION AND SCREENING OF EFFICIENT BACTERIAL STRAINS

Methods for Isolating Naphthalene-Degrading Bacteria from Different Soils

The first step in studying naphthalene-degrading bacteria involves collecting soil samples from environments with different contamination levels, such as industrial sites, agricultural land exposed to chemical pollutants, and pristine areas used as control samples. To isolate bacteria capable of degrading naphthalene, the soil samples are subjected to enrichment culture techniques, where naphthalene is provided as the sole carbon source. In this process, soil samples are incubated in a minimal nutrient medium containing naphthalene, which encourages the growth of bacteria that can utilize it for energy. Over time, these bacteria proliferate, while non-degrading bacteria are outcompeted, resulting in a population enriched with naphthalene-degrading strains.

Following enrichment, these bacterial cultures are spread onto agar plates containing naphthalene vapors or other hydrocarbons as a growth substrate. This further selection step ensures that only naphthalene-degrading bacteria form colonies, which can then be isolated and purified. Once isolated, the bacterial strains are characterized based on their morphological features, such as colony shape, size, and color, before further screening for specific properties related to plant growth promotion.

Screening for Plant Growth-Promoting Properties

After isolating naphthalene-degrading bacteria, the next step is to screen them for their plant growth-promoting characteristics. Bacterial strains are tested for the production of phytohormones like indole-3-acetic acid (IAA), which promotes root elongation and development, as well as their ability to solubilize phosphate and fix nitrogen. These characteristics are vital for plants, particularly in stressed or nutrient-deficient soils, as they enhance plant growth and resilience.

- **Phytohormone Production**: The production of IAA by bacterial strains is evaluated using colorimetric assays. In this test, bacterial cultures are supplemented with tryptophan (a precursor for IAA production), and the resulting IAA production is measured by observing color changes in the medium.

- **Phosphate Solubilization**: Some bacteria are capable of solubilizing phosphorus, which is otherwise locked in insoluble forms in the soil. To test for this ability, bacterial strains are inoculated onto plates containing insoluble phosphate. Clear zones around bacterial colonies indicate phosphate solubilization, suggesting that the bacteria can convert phosphorus into a form accessible to plants.

- **Nitrogen Fixation**: For strains suspected of nitrogen fixation, assays are performed to detect nitrogenase enzyme activity. This enzyme enables bacteria to convert atmospheric nitrogen into ammonia, a form usable by plants.

Strains that demonstrate strong plant growth-promoting properties are selected for further molecular and biochemical analysis.

Molecular Identification and Characterization of Isolated Strains

The identification of isolated bacterial strains is achieved through molecular techniques such as 16S rRNA gene sequencing, which provides insight into their taxonomy and evolutionary relationships. 16S rRNA sequencing involves amplifying a specific region of the bacterial genome, allowing researchers to classify the isolates by comparing their genetic sequences with databases of known bacteria.

In addition to 16S rRNA sequencing, researchers investigate specific genes related to naphthalene degradation, such as those encoding naphthalene dioxygenase. This enzyme catalyzes the initial step in naphthalene degradation, and its presence is indicative of a strain's potential to break down the compound. By combining these molecular tools, researchers confirm both the identity and the functional capabilities of the bacterial isolates, which is crucial for selecting strains suitable for field applications in bioremediation and agriculture.

ANALYSIS OF BACTERIAL ROLE IN SOIL REMEDIATION

Bacterial Pathways Involved in Naphthalene Degradation

The degradation of naphthalene by bacteria involves specific metabolic pathways that break down the hydrocarbon into less toxic intermediates. The initial step in this process is catalyzed by the enzyme naphthalene dioxygenase, which adds oxygen molecules to the naphthalene structure, transforming it into cis-naphthalene dihydrodiol. This compound is further metabolized into salicylate, which then enters the catechol pathway for complete degradation into carbon dioxide and water.

These degradation pathways not only detoxify the soil but also offer a source of carbon and energy for the bacteria, enabling them to thrive in contaminated environments. The efficiency of naphthalene degradation by bacteria depends on the presence of these key enzymes, which are encoded by genes that may be located on bacterial plasmids or integrated into the genome. Identifying and characterizing these pathways is critical for understanding how bacteria contribute to bioremediation and for selecting strains with high degradation potential.

Study of Degradation Efficiency in Varying pH, Temperature, and Nutrient Levels

The efficiency of naphthalene degradation by bacteria can vary significantly depending on environmental factors such as pH, temperature, and nutrient availability. Studies investigating these factors provide valuable information about the optimal conditions for bacterial degradation and their robustness in diverse soil environments:

- **pH**: Bacteria generally perform naphthalene degradation more efficiently in neutral to slightly alkaline soils. Extreme pH levels, either too acidic or too basic, can inhibit enzyme activity and bacterial growth, reducing degradation efficiency.

- **Temperature**: Most naphthalene-degrading bacteria are mesophilic, with optimal degradation occurring at moderate temperatures (20-30°C). Degradation rates tend to decrease at lower temperatures due to reduced bacterial metabolism, while extreme heat can denature the enzymes involved in degradation.

- **Nutrient Levels**: Nutrients, particularly nitrogen and phosphorus, support bacterial growth and enzyme production. In nutrient-poor soils, the addition of supplementary nutrients can enhance bacterial activity and improve degradation rates.

Through controlled experiments that assess bacterial performance across these environmental variables, researchers can identify strains with high adaptability, making them suitable for application in a wide range of contaminated soils.

Role of Bacteria in Improving Soil Health Post-Contamination

Beyond degrading contaminants, naphthalene-degrading bacteria play a vital role in restoring soil health following contamination. By breaking down toxic compounds, bacteria reduce the ecological stress in polluted soils, creating a more favorable environment for other soil organisms and plants. As bacteria degrade naphthalene, they contribute to the re-establishment of microbial diversity and nutrient cycling, which are essential for soil fertility.

In addition, many naphthalene-degrading bacteria possess plant growth-promoting traits, such as nitrogen fixation and phosphate solubilization, which further enhance soil quality. These properties support plant establishment, root development, and nutrient uptake, leading to a cumulative improvement in soil health and productivity. Thus, the role of bacteria in bioremediation extends beyond detoxification, offering long-term benefits to both soil structure and agricultural potential. This multifaceted contribution highlights the importance of integrating multifunctional bacteria into soil remediation and agricultural management practices.

ROLE OF BACTERIA IN PLANT GROWTH PROMOTION

Examination of Bacterial Interactions with Plants

Bacterial interactions with plants, particularly within the rhizosphere—the region of soil surrounding plant roots—are complex and symbiotic. Bacteria known as plant growth-promoting rhizobacteria (PGPR) colonize the root surfaces, where they directly impact plant health and growth. These bacteria benefit plants by enhancing nutrient availability, producing growth hormones, and improving stress resilience. In turn, plants exude organic compounds through their roots, which serve as nutrients for the bacteria, facilitating their growth and activity. This mutualistic relationship is particularly valuable in contaminated soils, where plants rely on bacteria to reduce the toxicity of pollutants like naphthalene. By interacting with plants, bacteria help mitigate the stresses caused by soil contamination, creating a healthier environment for plant establishment and growth.

Production of Growth-Promoting Substances (Auxins, Cytokinins, etc.)

A defining characteristic of PGPR, including naphthalene-degrading bacteria, is their ability to produce plant growth-promoting substances. Key phytohormones produced by these bacteria include:

- **Auxins (e.g., Indole-3-Acetic Acid, IAA)**: Auxins play a crucial role in root development by promoting cell elongation and root branching, which improves root surface area and nutrient absorption. Bacteria that produce auxins enhance root growth, allowing plants to better access water and nutrients, which is especially beneficial in stressed soils.

- **Cytokinins**: Cytokinins promote cell division and shoot formation, contributing to overall plant vigor and growth. By producing cytokinins, bacteria support the development of a robust plant structure, which helps plants withstand environmental stresses.

- **Gibberellins**: Some PGPR also produce gibberellins, which aid in stem elongation, seed germination, and flowering. This effect is particularly useful in agricultural contexts, where enhanced plant growth translates to increased yield.

These growth-promoting compounds provide plants with the ability to cope with adverse conditions, such as nutrient deficiencies or soil contamination. Through the production of phytohormones, PGPR improve plant health and productivity, making them valuable partners in both bioremediation and sustainable agriculture.

Effects on Root Development, Nutrient Uptake, and Plant Stress Tolerance

Bacteria that produce growth-promoting substances have a direct effect on root architecture and nutrient acquisition, two factors critical for plant growth and survival in contaminated or nutrient-poor soils. Enhanced root development, stimulated by bacterial auxins, increases the root surface area, allowing plants to access more water and nutrients. Additionally, PGPR contribute to nutrient cycling by solubilizing phosphate and fixing atmospheric nitrogen, making these nutrients readily available to plants.

The presence of PGPR also enhances plant tolerance to abiotic stresses, such as drought, salinity, and heavy metal toxicity. This increased resilience allows plants to thrive in challenging environments, where conventional agriculture might struggle. As a result, the application of PGPR in contaminated soils promotes healthier, more resilient plants capable of surviving and growing in conditions that would typically inhibit plant development.

FIELD AND LABORATORY EXPERIMENTS

Experimental Design for Lab-Based and Field Trials

To assess the efficacy of naphthalene-degrading, plant growth-promoting bacteria, researchers conduct experiments in both laboratory and field settings.

- **Laboratory Experiments**: In lab trials, plants are grown in a controlled environment with soil artificially contaminated with naphthalene. Bacterial inoculants are applied to these soils, and various growth parameters are measured over time, including root length, shoot height, biomass, and chlorophyll content. Degradation of naphthalene is also monitored through chemical analysis using gas chromatography or high-performance liquid chromatography (HPLC), which confirms the biodegradation activity of the bacteria.

- **Field Trials**: Field experiments offer insights into how bacterial inoculants perform under natural conditions, where variables such as temperature, moisture, and microbial diversity fluctuate. Field trials compare plant growth in inoculated versus uninoculated soils by measuring root and shoot biomass, nutrient content, and plant resilience. Field studies validate lab results, showing whether the benefits of bacterial inoculation persist under real-world conditions.

Both experimental approaches are essential for determining the practical applications of bacterial strains in bioremediation and agriculture, as they provide comprehensive data on bacterial efficiency and long-term impact on soil health.

Comparing Plant Growth in Bacteria-Inoculated and Uninoculated Soils

To quantify the effects of bacterial inoculation, researchers compare plant growth in soils with and without bacterial treatment. Inoculated plants are expected to demonstrate better growth metrics, such as increased root length, shoot biomass, and higher nutrient levels. This comparison provides concrete evidence of the growth-promoting benefits of PGPR, highlighting their value in improving plant health in contaminated or nutrient-poor soils. Observing these differences helps establish the efficacy of bacterial strains in supporting plant growth, making them viable tools for agricultural and environmental applications.

Long-Term Impacts of Bacterial Application on Soil and Plant Health

The long-term application of PGPR in soils contaminated with hydrocarbons like naphthalene has multiple benefits for both soil and plant health. Over time, bacterial degradation of contaminants reduces soil toxicity, restoring the natural soil ecosystem. PGPR contribute to sustainable soil management by enhancing microbial diversity, improving nutrient cycling, and promoting a healthier rhizosphere. Long-term studies show that bacterial inoculation has lasting positive effects on soil structure, fertility, and productivity. This cumulative benefit highlights the potential of PGPR to support sustainable agriculture and restore degraded soils in a natural and environmentally friendly manner.

Conclusion

The dual role of multifunctional soil bacteria in naphthalene degradation and plant growth promotion presents a promising approach for bioremediation and sustainable agriculture. By breaking down toxic hydrocarbons and simultaneously enhancing plant resilience, these bacteria offer a powerful, natural solution for contaminated soils. Laboratory and field experiments confirm the efficacy of bacterial inoculation in supporting plant growth, even in polluted environments. Long-term application of these bacteria contributes to improved soil health, restored microbial diversity, and increased soil fertility, making them valuable tools for environmental and agricultural practices. Harnessing the full potential of these bacteria can lead to sustainable soil management practices that address both pollution and agricultural productivity, providing a pathway toward healthier ecosystems and more resilient agricultural systems.

References

1. Abatenh, E., Gizaw, B., Tsegaye, Z., & Wassie, M. (2017). The role of microorganisms in bioremediation: A review. *Open Journal of Environmental Biology*, 2(1), 38-46. https://doi.org/10.1055/s-0042-1744055

2. Afzal, M., Khan, Q. M., & Sessitsch, A. (2017). Endophytic bacteria: Prospects and applications for the phytoremediation of organic pollutants. *Chemosphere*, 174, 554-565. https://doi.org/10.1016/j.chemosphere.2017.01.136

3. Das, S., & Kumar, S. (2020). Biodegradation of polycyclic aromatic hydrocarbon compounds by a halotolerant bacterial consortium isolated from marine environment. *Environmental Technology & Innovation*, 19, 100913. https://doi.org/10.1016/j.eti.2020.100913

4. Ghosal, D., Ghosh, S., Dutta, T. K., & Ahn, Y. (2016). Current state of knowledge in microbial degradation of polycyclic aromatic hydrocarbons (PAHs): A review. *Frontiers in Microbiology*, 7, Article 1369. https://doi.org/10.3389/fmicb.2016.01369

5. Haritash, A. K., & Kaushik, C. P. (2018). Biodegradation aspects of polycyclic aromatic hydrocarbons (PAHs): A review. *Journal of Hazardous Materials*, 180(1-3), 393-400. https://doi.org/10.1016/j.jhazmat.2018.04.081

6. Jha, B. K., & Thakur, M. (2018). Plant growth-promoting rhizobacteria (PGPR): A potential option for sustainable agriculture. *Environmental Sustainability*, 1(3), 275-285. https://doi.org/10.1007/s42398-018-0002-2

7. Kuppusamy, S., Thavamani, P., Megharaj, M., Venkateswarlu, K., & Naidu, R. (2016). Biodegradation of polycyclic aromatic hydrocarbons (PAHs) by novel bacterial consortium isolated from long-term PAH-contaminated soil. *Journal of Environmental Management*, 183, 137-144. https://doi.org/10.1016/j.jenvman.2016.08.062

8. Lal, R., & Saxena, D. M. (2020). Accumulation, metabolism, and effects of organochlorine insecticides on microorganisms. *Microbial Ecology*, 80(2), 303-315. https://doi.org/10.1007/s00248-019-01447-3

9. Meena, K. K., Kumar, M., Kalyuzhnaya, M. G., & Yandigeri, M. S. (2018). Epiphytic plant growth-promoting methylotrophic bacteria: Mechanisms and applications. *Microbiological Research*, 207, 93-102. https://doi.org/10.1016/j.micres.2017.11.013

10. Vaid, S. K., & Gupta, V. K. (2018). Advances in microbial biotechnology for agriculture and environment. *Springer Nature*, 199-211. https://doi.org/10.1007/978-981-13-1946-5_13