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**Applied and Environmental Microbiology | MICR 3213**

**Phenol Degraders**

**October 30,2024**

**Department of Basic Medical Science**

**Title:** Isolation and Growth of Aerobic Phenol Degrading Bacteria

**Aim:** To isolate naturally occurring soil bacterial strains capable of degrading phenol and evaluate their growth in the presence of different concentrations of phenol and other aromatic compounds.

**Abstract:**

This experiment was aimed at isolating and charactering soil bacterial strains capable of phenol degradation from samples collected at the Riverton landfill and the UWI, Mona campus. The collected samples were cultured in mineral salt medium (MM) containing phenol as the sole carbon source. The number of isolates and their colony’s morphological characteristics were tabulated. The isolates were further tested for growth on MM with varying phenol concentrations (100-1500 mg/L). Selected colonies were Gram stained and viewed microscopically to further identify them.

The results indicated varied bacterial growth across different phenol concentrations, with colony descriptions recorded for morphological differentiation. Growth at high phenol concentrations indicated potential candidates for bioremediation. The isolates that thrived in higher phenol levels suggested promising metabolic pathways for phenol degradation, with Gram-positive and Gram-negative morphologies observed. The ecological significance of phenol-degrading bacteria in mitigating pollution, with isolates like *Pseudomonas putida* and *Acinetobacter calcoaceticus* suggested based on their known resilience in diverse, phenol-laden environments. These findings support the potential use of native microbial strains in bioremediation strategies for contaminated sites, contributing to more sustainable environmental management practices.

**Introduction:**

Phenol and its derivatives are prevalent pollutants in the environment due to their widespread use in industrial processes, including chemical manufacturing, plastics, and pharmaceuticals. Despite their utility, phenolic compounds are highly toxic to aquatic life and pose significant environmental hazards when improperly managed, they are often even carcinogenic to humans. The biodegradation of phenol by microorganisms offers a sustainable and effective means of pollution control, leveraging naturally occurring bacteria capable of metabolizing phenol as a carbon source. This not only eliminates secondary pollutants generation, microbial bioremediation of phenol and phenolic compounds are often times cheaper.

Phenol-degrading bacteria have demonstrated significant potential for environmental cleanup due to their metabolic versatility and adaptive capabilities. This experiment was designed to isolate soil bacteria capable of degrading phenol from two distinct environments—the Riverton landfill and the UWI, Mona campus—to assess their growth and degradation efficiency in media containing phenol at various concentrations.

Therefore, this research is aimed at characterizing the isolated strains through morphological, biochemical, and Gram staining analyses and evaluate their tolerance levels to phenol. Understanding the behavior and characteristics of these phenol-degrading microorganisms is essential for developing effective bioremediation techniques, which are safer and often more economical than conventional chemical methods that are prevalent in todays industry.

**Method:** As adapted from MICR 3213 [BC31M] Applied and Environmental Microbiology Laboratory Manual.

**Results:**

**TABLE SHOWING THE NUMBER OF ISOLATES TAKEN FROM MM MEDIUM PLATED SUPPLEMENTED WITH PHENOL AND THE COLONY MORPHOLOGY**

|  |  |  |
| --- | --- | --- |
| Phenol Group | # of isolates and the plate | Colony description |
| 1 | 2 (pink and white) CB & SA  Streak 2(Monday)  White colonies | 1- Pink, Circular, Glossy, Smooth edge, drop like elevation  2-offwhite, circular, small, drop like elevation  Streak 2 Monday  Smooth, small round White colonies |
| 2 | 2 (orange& white) MWSS | 1- Small, round, orange colonies with smooth margins  2- Off-white colonies with wavy margins and convex elevation |
| 3 | 1(pink) CC & JM | 1 - small, pink, smooth, circular, flat colonies |
| 4 | 1 (off-white) SAMR | Small round colonies off white in colour with smooth surfaces and margins and drop like elevations |
| 5 | 2 (pink&white) KC AJ |  |
| 6 | 1 white colony + 1 pink MOCH |  |
| 7 | 1 off white colony KEV | Small round off-white colonies with smooth margins and convex elevation |
| 8 | 1 pink isolate SDC | Pink isolate - smooth round and convex, tiny and circular. |

**TABLE SHOWING GROWTH OF MICOORGANISMS WITH DIFFERING CONCENTRATIONS OF PHENOL**

|  |  |
| --- | --- |
| **Concentration of Phenol mG/L** | **Colony Growth** |
| 100 MOCH | Small, Off-white coccid shape, isolated growth. |
| 300 MOCH | Small, Off-white, coccid shape, colony has a grape like growth. |
| 1000 MOCH | Small, Off-white, coccid shape, isolated growth. |
| 1500 MOCH | Small, Off-white coccid shape, cluster colony growth |
| 100 SA + CB | Very small, off white, coccid shaped |
| 300 SA + CB | Very small, off white, coccid shaped |
| 1000 SA + CB | Very small, off white, coccid shaped |
| 1500 SA + CB | Very small, off white, coccid shaped |

**TABLE SHOWING RESULTS OF GRAM STAINING AND MICROSCOOPIC OBSERVATION**

|  |  |
| --- | --- |
| **Sample** | **Observation** |
| MOCH | Gram +ve rod shaped at x1000 mag |
| SA + CB | Gram -ve cocci shaped at x1000 mag |

**Discussion:**

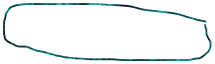
The collection of the soil samples from different locations in this case; Riverton landfill and the UWI, Mona campus, where high pollutant exposure is likely, functions to ensure that a variety of microorganisms are included in the sample. This step is crucial as these sites are more likely to harbor microorganisms adapted to degrading toxic compounds like phenol, especially those from the Riverton Landfill. The preparation of 0.5% agar medium and the use of MM enriched with essential minerals and phenol ensures that only the desired/selected phenol-degrading bacteria are allowed to grow in the media as they would be the only ones capable of using the phenol carbon source for growth. This acts to inhibit the growth of bacteria which are unable to degrade phenol. Incubating the plates under aerobic condition further facilitate the proliferation of phenol-degraders, as most know phenol-degraders

Tabulating the number of isolates observed on the MM agar (step 11) functions as a means to quantify the potential of the soil sample in terms of microbial abundance of which, some may be capable of phenol degradation. The streaking of the colonies onto MM plates with and without phenol (step 12) helps in identifying true phenol-degrading strains versus those that may grow on alternative substrates present in the original medium, as in the second follow up, students were advise to only chose the isolate stain that grew on the plate with phenol and did not grow on the plate without phenol. Incubation for 120 hours ensures sufficient time for observable growth (step 13). Describing colony morphology (step 14) aids in differentiating the strains based on their visual characteristics.

In the second follow up session, selecting the colonies that grew only on the plate containing phenol, confirms that phenol was necessary substrate for its growth. The staining of the microorganism isolated functions as a means of further identifying which specific phenol-degrader was present on the medium. The differing concentrations of phenol was used to assess the tolerance level and metabolic capabilities of the bacterial strain isolated. If growth is observed at 1000 or 1500 mg/L then the isolate possesses significant bioremediation qualities (Morones-Esquivel et al., 2022).

**Questions:**

1. Phenol is a significant player in industries; however, it is toxic and has detrimental effects on the environment and aquatic life due to run off. As a result, phenol degraders are used to counteract these effects as they are often cheaper and safer as it pertains to the generation of secondary pollutants than their physiochemical counterparts (Malhotra et al., 2021). Two phenol degraders may have been present; *Pseudomonas putida* and *Acinetobacter calcoaceticus.* In areas where the type of waste present is as diverse as at the Riverton Landfill, phenol degraders such as *Pseudomonas putida* may be present as it thrives in these environments, where chemical and industrial waste containing hydrocarbons and phenolic compounds may be present. As it pertains to *Acinetobacter calcoaceticus*, this bacteria is often present in soil or water bodies that receive a low level of runoff, this is due to its adaptability to its environments.
2. The word Recalcitrance refers to the resistance of certain organic compounds to degradation by natural biological processes (*Recalcitrant*, n.d.). This is often a result of their complex structures. An example of a recalcitrance compound would be polychlorinated biphenyls (Legron-Rodriguez, 2013).
3. Chlorinated compounds are more recalcitrant due to the creation of strong carbon-chlorine bonds which are more difficult to cleave. Also this mat hinder enzymatic processes of microbial degradation, additionally, they are less soluble in water (Nikel et al., 2013).

Soil/Water Contamination

↓

Isolation of Microorganism

↓ (Selected for its ability to degrade phenol)

Culturing in Controlled Environment

↓

Inoculation into Contaminated Site

↓ (Microbes begin degrading phenol into intermediate compounds)

Biotransformation

↓ (Further breakdown into non-toxic end products like CO2 and water)

Monitoring and Maintenance

↓ (Ensures the ideal conditions are present for microbial activity)

Restoration of Ecosystem

**Conclusion:**

This experiment successfully isolated and characterized bacteria from soil samples capable of degrading phenol, a common environmental pollutant. The results highlight the potential of these microbial strains for bioremediation applications. Evaluation of the isolated strains' degradation efficiency in real-world contaminated environments could be done to combat phenol pollution. Also optimization of growth conditions and development of bioremediation strategies using these strains could result in more economical bioremediation strategies.

**References**

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