

Isolation and Propagation of Methomyl-Degrading Bacteria from Pesticide-Polluted Land

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Abstract

Methomyl is a widely used carbamate pesticide in horticulture, valued for its broad-spectrum insecticidal properties and rapid action. Despite its efficacy in pest control, methomyl poses significant environmental and human health risks due to its persistence in soil and toxicity to non-target organisms. Residual methomyl can contaminate soil and water bodies, leading to bioaccumulation in ecosystems and potential adverse effects on human health. This study aimed to isolate and identify indigenous bacteria from shallot farm soils in Central Java, Indonesia, capable of utilizing methomyl as a carbon and energy source, thereby facilitating its biodegradation. Soil samples from methomyl-treated fields were collected and subjected to enrichment culture techniques using Minimal Salt Medium with varying methomyl concentrations to select for resistant and metabolizing bacteria. Four distinct bacterial isolates (RW, RC, RY, and RR) were recovered, showing morphological diversity and varied growth responses to methomyl exposure. Among these, isolates RW and RC exhibited superior adaptability and growth performance, with the filamentous isolate RC demonstrating the highest growth potential, indicative of its robust methomyl degradation capacity. These findings emphasize the promising role of native soil bacterial communities in bioremediation strategies to mitigate pesticide pollution. Further research is warranted to elucidate the metabolic pathways involved, quantify degradation efficiency, and assess the practical application potential of these isolates in contaminated environments.

Keywords: *Methomyl, Pesticide, Biodegradation, Soil Bacteria, Environmental Pollution*

INTRODUCTION

Agricultural productivity is critically dependent on effective pest management strategies, with pesticides playing an essential role in protecting crops from harmful insect infestations and disease vectors. Among the numerous pesticides employed globally, methomyl, a carbamate-based insecticide, has garnered widespread use in horticultural crops such as shallots, tomatoes, and various vegetables due to its broad-spectrum activity and rapid insecticidal effect (Siahaya, 2021). Methomyl functions by inhibiting the acetylcholinesterase enzyme, which is crucial for nerve impulse transmission in insects. By hydrolyzing acetylcholine, the pesticide disrupts nerve function, leading to paralysis and death of targeted pests, making it an effective agent for crop protection. Methomyl residues in the soil are toxic to non-target organisms, degrade soil quality, and contaminate water in agricultural areas (Boff et al., 2022). Continuous contamination led to bioaccumulation and biomagnification in the animals involved in the food chain. Meanwhile, effects experienced by humans exposed to methomyl include muscle spasms, pallor, blurred vision, low blood pressure, and loss of nerve reflexes. Chronic effects can cause teratogenic effects on the reproductive process (Bhandari et al., 2020).

However, the extensive and repeated application of methomyl in agricultural settings raises concerns about its environmental persistence and adverse impacts on non-target organisms. Methomyl residues often accumulate in soil, adversely affecting soil microbial communities,

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altering soil chemistry, and reducing overall soil fertility (Boff et al., 2022). Moreover, its high-water solubility increases the risk of leaching into groundwater, thereby contaminating aquatic ecosystems and threatening biodiversity. These environmental repercussions are compounded by the potential for bioaccumulation and biomagnification through the food chain, posing risks to both wildlife and humans.

In humans, exposure to methomyl, whether acute or chronic, can lead to a range of health issues, including neurological symptoms such as muscle spasms, blurred vision, and reduced nerve reflexes, as well as cardiovascular disturbances like hypotension (Bhandari et al., 2020). Prolonged exposure is particularly worrisome, with studies indicating teratogenic effects and disruptions in reproductive health. The dual challenge of maintaining crop protection and safeguarding environmental and public health necessitates the development of sustainable pesticide management practices.

One promising avenue to address this challenge is through bioremediation, the use of living organisms, particularly microorganisms, to degrade or detoxify environmental contaminants. Soil microorganisms, including bacteria and fungi, possess diverse enzymatic systems capable of transforming complex and persistent compounds like methomyl into less toxic or inert substances. These microbes metabolize pesticides by breaking chemical bonds, altering functional groups, and ultimately mineralizing the compounds into carbon dioxide, water, and other harmless end products (Omotayo & Omotayo, 2024). The identification and utilization of indigenous bacteria that thrive in pesticide-contaminated soils offer a sustainable and eco-friendly solution to mitigate pesticide residues.

In this context, this study focuses on the isolation and characterization of methomyl-degrading bacteria from shallot farm soils in Central Java, Indonesia. By assessing the morphological traits and growth viability of bacterial isolates in methomyl-enriched media, this research aims to lay the foundation for developing microbial-based bioremediation agents tailored for methomyl-contaminated environments. Such strategies hold promise for enhancing soil health, reducing environmental pollution, and promoting safer agricultural practices.

LITERATURE REVIEW

Pesticides are chemical agents used to control pests that damage crops, including insects, weeds, and microorganisms (Prajawahyudo et al., 2022). Methomyl, a carbamate insecticide introduced in 1968, is widely used for its broad-spectrum effectiveness against various insect pests in horticulture (Sharma et al., 2019). It works by inhibiting acetylcholinesterase, disrupting nerve impulses, and leading to insect death.

Despite its efficacy, methomyl poses environmental and health risks. It is toxic to non-target organisms, including mammals and aquatic life, and can persist in soils at concentrations ranging from 1 µg/kg to 480 µg/kg (Riedo et al., 2023). Methomyl residues often accumulate in the root zone, affecting soil quality and microbial communities (Faraj et al., 2024). Human exposure can cause acute symptoms such as muscle spasms and blurred vision, with chronic exposure linked to reproductive effects (Bhandari et al., 2020).

Methomyl degrades through abiotic processes like hydrolysis, which breaks ester bonds, producing less harmful compounds such as methomyl oxime and acetic acid (Catania, 2020). However, microbial biodegradation is a more effective pathway for complete detoxification. Many bacteria utilize methomyl as a carbon or energy source, breaking it down into non-toxic end products. Known methomyl-degrading bacteria include *Photobacterium ganghwense*, *Paracoccus* sp., *Stenotrophomonas maltophilia*, *Pseudomonas* sp., and *Bacillus cereus* (Lin et al., 2020; Guerrero Ramírez et al., 2023). For example, *Stenotrophomonas maltophilia* has demonstrated growth on methomyl-containing media, while *Pseudomonas* spp. can degrade up to 70% of methomyl in

contaminated water ([Mohamed, 2009](#); [Xu et al., 2009](#)).

Biodegradation efficiency depends on bacterial species, environmental factors, and methomyl concentration. [Mohamed \(2009\)](#) reported that 100 ppm methomyl was degraded within 10 days at pH 7 and 30°C. Given soil complexity, isolating native bacteria adapted to local pesticide conditions is vital for effective bioremediation. This study focuses on identifying such bacteria from methomyl-contaminated shallot farm soils in Central Java to develop sustainable pesticide mitigation strategies.

RESEARCH METHOD

Sampling site and soil collection

Soil samples were collected from a shallot farm located in Sendang Village, Kendal Regency, Central Java, Indonesia, known for routine methomyl pesticide applications. The site was chosen due to its history of methomyl use, which likely influenced microbial populations adapted to degrade this pesticide. Three distinct sampling points were selected within the farm to capture spatial variability in soil characteristics and microbial communities.

Sample preparation and dilution

The three soil samples were composited into a homogenized bulk sample to provide a representative microbial population for the study. Ten grams of this composite soil were subjected to serial tenfold dilutions (from 10^{-1} to 10^{-5}) to reduce microbial density and facilitate isolation of distinct bacterial colonies. This dilution approach allows the selective growth of bacteria with varied tolerance levels to methomyl when cultured on selective media.

Enrichment and isolation of bacteria

To isolate methomyl-degrading bacteria, 100 μL of the 10^{-5} dilution was inoculated onto Minimal Salt Medium (MSM) plates supplemented with four different methomyl concentrations: 0 ppm (control), 25 ppm, 50 ppm, and 75 ppm. MSM is a nutrient-limited medium designed to force bacteria to utilize methomyl as a primary carbon and energy source, thereby enriching for organisms capable of metabolizing the pesticide. Plates were incubated at 30°C for 14 days to allow for sufficient bacterial growth, especially of slow-growing or stressed populations exposed to methomyl.

After incubation, distinct bacterial colonies were counted and carefully picked based on morphological differences such as color, shape, texture, and colony elevation to ensure diversity among isolates. Selected colonies were streaked onto Nutrient Agar (NA) plates to obtain pure cultures. These pure isolates were then stored on agar slants at 4°C for further analyses.

Morphological characterization

Isolated bacteria were characterized morphologically by observing colony appearance, including color, form, texture, elevation, and edge. This characterization assists in distinguishing between bacterial species or strains, providing preliminary identification and grouping.

Viability and growth assessment in liquid culture

To evaluate bacterial growth and methomyl degradation potential, pure isolates were cultured in Nutrient Broth (NB) supplemented with varying methomyl concentrations (25, 50, 75, and 100 ppm). The cultures were incubated in a shaker incubator at 30°C with shaking at 120 rpm to ensure aeration and homogeneous distribution of nutrients and pesticides. Bacterial growth was monitored by measuring optical density (OD) at 600 nm using a spectrophotometer at 24, 48, and 72 hours post-inoculation. Optical density is a proxy for cell density, where higher OD values

indicate increased bacterial proliferation. Growth curves generated from these measurements provided insight into the ability of each isolate to survive and multiply in methomyl-contaminated environments, suggesting their potential for pesticide degradation.

FINDINGS AND DISCUSSION

Morphological characteristics of isolates

The study successfully isolated four distinct bacterial strains from methomyl-treated shallot soils, labeled as RW, RC, RY, and RR. Each exhibited unique morphological features, summarized in Table 1.

Table 1. Morphological characteristics of the obtained isolate

No.	Code	Color	Shape	Texture	Elevation	Edge
1	RW	White	Round	Shiny	Convex	Entire
2	RC	Cream	Filamentous	Shiny	Convex	Entire
3	RY	Yellow	Round	Shiny	Convex	Entire
4	RR	Red	Round	Shiny	Convex	Entire

Isolate RW displayed the highest colony count on MSM across all methomyl concentrations tested (Figure 1), demonstrating robust adaptability to pesticide stress. This isolate's white, round, shiny colonies with convex elevation and smooth edges suggest a typical aerobic heterotrophic bacterium with strong metabolic capabilities. Isolate RC, characterized by a filamentous colony morphology, grew optimally at 50 and 100 ppm methomyl, indicating a high tolerance to pesticide toxicity. Filamentous growth may confer enhanced survival mechanisms such as biofilm formation or extracellular polymer production, aiding resistance to toxic compounds (Ma et al., 2024). In contrast, isolates RY and RR showed limited growth and lower colony counts, particularly at higher methomyl concentrations, suggesting less effective adaptation or degradation capability. Isolate RR was the least abundant and was only recovered at 25 and 50 ppm methomyl.

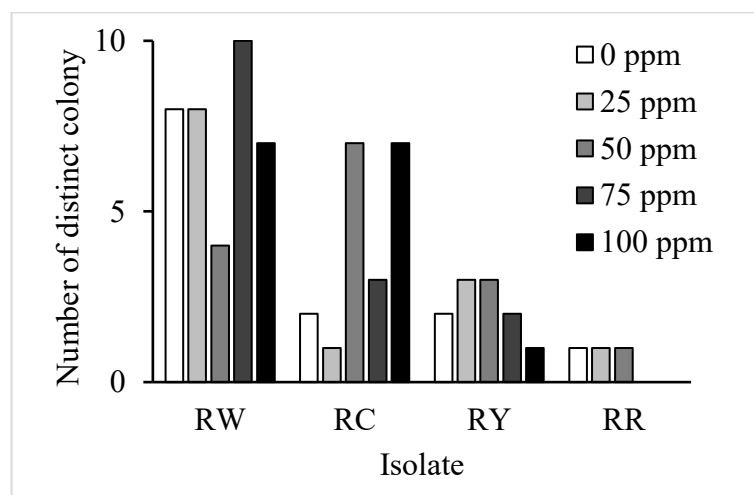


Figure 1. Number of distinct colonies in the Minimum Salt Medium (MSM) with various methomyl concentrations

Growth viability and adaptability in methomyl-containing media

Figure 2 illustrates the optical density (OD₆₀₀) profiles of the four isolates cultured in liquid media with different methomyl concentrations over 24, 48, and 72 hours. At 24 hours, isolate RC demonstrated the highest growth rate at 50 ppm methomyl (OD 0.53), followed closely by RW at 75 ppm (OD 0.513) and 100 ppm (OD 0.44). The rapid growth of RC likely stems from its filamentous nature and extracellular polymer production, which may protect cells against methomyl toxicity. Isolates RY and RR exhibited lower OD values (0.129–0.138) at the same time point, indicating slower initial adaptation and growth in the pesticide environment.

After 48 hours, all isolates showed increased growth, with RC and RW reaching OD values close to 0.92, reflecting active metabolism and cell division despite methomyl presence. Isolate RY showed notable improvement at 50 ppm, increasing by 0.624 OD units, whereas RR remained the least vigorous (OD 0.331). By 72 hours, growth plateaued or slightly decreased, suggesting that the cultures had reached the stationary phase, possibly due to nutrient depletion or accumulation of toxic metabolites.

These growth patterns reflect the varying abilities of the isolates to tolerate and potentially metabolize methomyl. The superior performance of isolate RC, particularly in higher methomyl concentrations, positions it as a prime candidate for bioremediation applications.

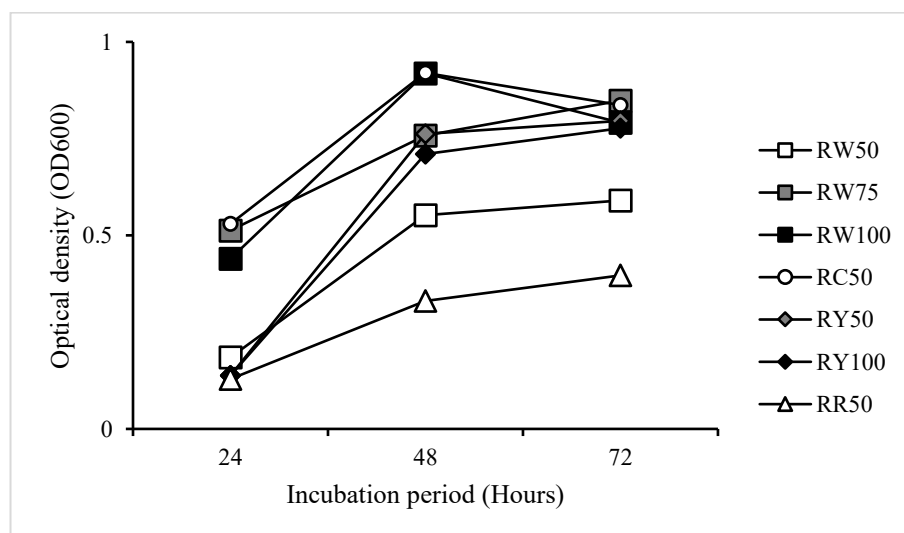


Figure 2. Optical density (OD₆₀₀) of obtained isolates in the 24, 48, and 72 hours of incubation periods. Letters indicate the isolate codes, and numbers indicate the methomyl concentration.

Implications for Biodegradation and Bioremediation

Microbial degradation of pesticides like methomyl involves enzymatic processes that transform toxic compounds into harmless substances through hydrolysis, oxidation-reduction, and other metabolic reactions (Guerrero Ramirez et al., 2023). The strong growth of isolates RW and RC in methomyl-enriched media suggests they possess such enzymatic systems capable of breaking down methomyl's chemical structure. The filamentous nature of isolate RC might facilitate extracellular polymer secretion, contributing to biofilm formation and enhanced resistance, which is advantageous for survival in contaminated soils. This trait is often associated with improved degradation efficiency and environmental resilience (Ma et al., 2024).

While isolates RY and RR showed weaker growth, they may still play auxiliary roles in microbial consortia involved in pesticide degradation, highlighting the importance of diverse microbial communities in soil health. Future work should focus on molecular identification, enzyme assays, and quantifying actual methomyl degradation rates by these isolates to confirm their

bioremediation potential and optimize their use in contaminated site management.

CONCLUSIONS

This study isolated four bacterial strains (RW, RC, RY, and RR) from methomyl-contaminated shallot farm soils. Among them, isolate RC showed the highest growth and adaptability in methomyl-containing media, indicating strong potential for pesticide degradation. Isolate RW also demonstrated notable adaptability, while RY and RR exhibited lower tolerance. These results highlight the promise of native soil bacteria as natural bioremediation agents for methomyl contamination. However, further research is needed to confirm their degradation abilities through molecular identification, enzyme characterization, and degradation efficiency tests. Additionally, testing in real soil conditions and exploring bacterial consortia will help optimize their application in environmental cleanup.

LIMITATIONS & FURTHER RESEARCH

These results highlight the promise of native soil bacteria as natural bioremediation agents for methomyl contamination. Several limitations should be acknowledged is needed to confirm their degradation abilities through molecular identification, enzyme characterization, and degradation efficiency tests. Additionally, testing in real soil conditions and exploring bacterial consortia will help optimize their application in environmental cleanup.

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