International Journal of Science and Research (IJSR)
ISSN: 2319-7064
Impact Factor 2025: 7.089

Isolation and Characterization of Pesticide Tolerant Bacteria and their Role in Enhancing Plant Growth in Pesticide Contaminated Soil

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Abstract: ***Soil contamination resulting from intensive pesticide use poses a significant threat to microbial diversity and soil functionality in agricultural systems all over the globe. This study focuses on the isolation and characterization of pesticide-tolerant bacteria from pesticide- contaminated soil and evaluates their potential role in plant growth promotion under laboratory conditions. Soil samples were collected from chilli cultivation fields in Walayar, Palakkad, Kerala, an area subjected to frequent pesticide use. Bacterial isolation was carried out using the spread plate technique on nutrient agar incorporated with monocrotophos at concentrations of 100 ppm and 200 ppm, followed by confirmation of tolerance at 300 ppm through streak plating. A representative isolate was purified and characterized based on morphological, microscopic, and biochemical properties. The isolate was identified as a Gram-negative, rod-shaped bacterium showing positive reactions for methyl red, oxidase, and catalase tests, and negative reactions for Voges–Proskauer, citrate utilization, and indole production. Evaluation of plant growth-promoting factors revealed positive ammonia production, while indole-3-acetic acid production and phosphate solubilization were not observed under the conditions tested. The ability of the isolate to grow across multiple pesticide concentrations indicates adaptive tolerance, while the selective expression of functional traits depicts a limited but potentially relevant role in nutrient cycling. These findings provide initial insight into the presence of bacterial populations which show resilience in pesticide-impacted soils and their possible contribution to maintaining soil functionality. Further studies involving molecular identification and quantitative analysis are required to better understand their ecological significance and potential applications in sustainable agriculture.***

Keywords: Pesticide tolerance, Monocrotophos, Soil bacteria, Plant growth-promoting traits, Ammonia production, Agricultural soil, PGPR

1. Introduction

Soil is a dynamic and biologically active system that plays a vital role in sustaining terrestrial ecosystems. It serves as a reservoir of diverse microbial communities that regulate essential ecological processes such as nutrient cycling, organic matter decomposition, and maintenance of soil fertility. Among these microorganisms, bacteria represent one of the most abundant and functionally significant groups due to their metabolic versatility and rapid response to environmental changes (Torsvik & Øvreås, 2002; van der Heijden et al., 2008). These microbial processes are crucial for maintaining soil productivity and ecological stability.

The intensification of agricultural practices has led to the extensive use of chemical pesticides to improve crop yield and protect plants from pests and diseases. While these compounds are effective in pest control, their excessive and continuous use has resulted in the accumulation of residues in soil ecosystems (Damalas & Eleftherohorinos, 2011). Pesticides can interact with soil components and exert toxic effects on non-target organisms, including beneficial soil microorganisms, thereby altering microbial diversity and activity which results in low yield and deterioration of soil health. (Aktar et al., 2009; Cycoń et al., 2010).

The response of soil microorganisms to pesticide exposure is complex and influenced by several environmental and chemical factors. While many microbial populations are sensitive to pesticide toxicity, certain bacteria develop adaptive mechanisms that enable them to survive under chemically stressed conditions (Imfeld & Vuilleumier, 2012). These adaptive responses may include enzymatic detoxification of pesticide compounds, efflux systems that remove toxic substances, and metabolic adjustments that support survival under stress (Singh et al., 2004).

Organophosphate pesticides, such as monocrotophos, are widely used due to their high effectiveness against a broad range of insect and pests. These compounds function primarily by inhibiting acetylcholinesterase, leading to disruption of nerve transmission in target organisms (Singh & Walker, 2006). However, their presence in soil creates a chemically stressful environment that affects microbial survival and activity. Despite these adverse conditions, certain bacterial populations exhibit tolerance and continue to function, indicating their adaptive potential in pesticide-contaminated environments (Cycoń et al., 2017).

Pesticide-tolerant bacteria play an important ecological role in maintaining soil functionality in contaminated environments. These microorganisms contribute to nutrient cycling, organic matter decomposition, and microbial balance, thereby supporting soil health even under stress conditions. In addition to tolerance, some bacteria possess plant growth-promoting (PGP) traits that enhance plant development through mechanisms such as nutrient mobilization and metabolite production (Glick, 2012; Bhattacharyya & Jha, 2012).

Plant growth-promoting traits such as indole-3-acetic acid (IAA) production, phosphate solubilization, and ammonia production are widely studied due to their importance in improving plant health and productivity. IAA influences root development and cell elongation, phosphate solubilization increases phosphorus availability, and ammonia production contributes to nitrogen cycling in soil (Patten & Glick, 2002; Rodríguez & Fraga, 1999). However, the expression of these traits is often strain-specific and influenced by environmental conditions. Under stress conditions, such as pesticide exposure, bacteria may exhibit selective expression of functional traits rather than a broad range of activities (Spaepen et al., 2007).

Despite growing interest in pesticide-tolerant microorganisms, there remains limited information on their functional characteristics in relation to plant growth promotion under pesticide-stressed conditions. Most studies have focused either on pesticide degradation or on plant growth-promoting traits under non-stressed environments, leaving a gap in understanding the combined role of tolerance and functionality.

The present study aims to isolate pesticide-tolerant bacteria from pesticide-contaminated agricultural soil and to characterize their morphological, microscopic, and biochemical properties. In addition, selected plant growth-promoting traits are evaluated to assess the functional potential of the isolate. This integrated approach provides insight into microbial adaptation and highlights the ecological significance of such bacteria in sustaining soil processes under pesticide-induced stress conditions

2. Materials and Methods

2.1 Study Area and Sample Collection

Soil samples were collected from chilli cultivation fields located in Walayar, Palakkad district, Kerala, India, an area subjected to regular pesticide application. Samples were collected from the rhizosphere region at a depth of approximately 7–10 cm using sterile tools to minimize contamination. Surface debris was removed prior to sampling, and the collected soil was transferred aseptically into sterile containers and transported to the laboratory for immediate testing.

2.2 Preparation of Soil Suspension

Approximately 1 g of soil sample was aseptically transferred into 9 mL of sterile distilled water to obtain an initial suspension. The mixture was mixed thoroughly to facilitate the release of microorganisms from soil particles evenly. Serial dilutions were prepared from this suspension for following isolation procedures.

2.3 Pesticide and Preparation of Pesticide-Containing Media

A commercial formulation of monocrotophos (36% SL) was used as the pesticide source which is also the same pesticide used in the field from where the sample was collected. A stock solution (1000 ppm) was prepared by diluting 1 mL of the pesticide in 1 L of sterile distilled water. From this stock, working concentrations of 100 ppm, 200 ppm, and 300 ppm were prepared using standard dilution procedures ($C_1V_1 = C_2V_2$).

The required volumes of the stock solution were added to molten nutrient agar after autoclaving and cooling to an appropriate temperature to prevent thermal degradation of the pesticide. The medium was mixed thoroughly to ensure uniform distribution and poured into sterile Petri plates.

2.4 Isolation of Pesticide-Tolerant Bacteria

Isolation of bacteria was performed using the spread plate technique on nutrient agar incorporated with monocrotophos at concentrations of 100 ppm and 200 ppm . Serial dilutions of 10^{-3} and 10^{-4} were selected for plating. 0.1 ml of each dilution was spread uniformly over the surface of the agar using a sterile glass spreader.The inoculated plates were incubated at 37°C for 24 hours. After incubation, distinct colonies were observed and selected for further analysis.

2.5 Further Screening for Pesticide Tolerance

Bacterial colonies exhibiting growth on pesticide-containing media were considered tolerant to monocrotophos. Selected colonies were further subjected to tolerance confirmation by streaking on nutrient agar plates incorporated with 300 ppm of the pesticide. Growth observed after incubation at 37°C for 24 hours was considered indicative of tolerance under higher pesticide stress (Imfeld & Vuilleumier, 2012).

2.6 Purification and Maintenance of Isolate

Selected bacterial colonies were purified using repeated streak plate techniques on nutrient agar until well-isolated colonies with uniform morphology were obtained. The purified isolate was maintained on nutrient agar slants and stored under refrigerated conditions for further characterization.

2.7 Morphological and Microscopic Characterization

Colony morphology was recorded based on characteristics such as shape, size, color, margin, elevation, and texture. For microscopic analysis, Gram staining was performed and the stained smears were observed under oil immersion to determine cell morphology and Gram reaction.

2.8 Biochemical Characterization

The bacterial isolate was subjected to standard biochemical tests, including methyl red (MR), Voges–Proskauer (VP), citrate utilization, indole production, oxidase, and catalase tests, following established microbiological protocols. Observations were recorded based on color changes or visible reactions indicative of specific metabolic activities.

2.9 Assessment of Plant Growth-Promoting Traits

2.9.1 Indole-3-Acetic Acid (IAA) Production

The isolate was inoculated into nutrient broth supplemented with L-tryptophan and incubated at 37°C for 24–48 hours. After incubation, the culture was centrifuged, and Salkowski's reagent was added to the supernatant. The development of pink coloration indicated IAA production (Gordon & Weber, 1951; Patten & Glick, 2002).

2.9.2 Phosphate Solubilization

Phosphate solubilization was assessed using Pikovskaya's agar. The isolate was spot inoculated onto the medium and incubated at 37°C for 48–72 hours. The formation of a clear halo zone around the colony indicated phosphate solubilization (Pikovskaya, 1948; Sharma et al., 2013).

2.9.3 Ammonia Production

The isolate was inoculated into peptone water and incubated at 37°C for 24–48 hours. After incubation, Nessler's reagent was added, and the development of yellow to brown coloration was recorded as a positive result for ammonia production (Cappuccino & Sherman, 2014).

2.10 Experimental Approach

All experiments were performed under aseptic conditions. Observations from morphological, biochemical, and plant growth-promoting assays were recorded qualitatively based on visible changes. The overall experimental design focused on evaluating pesticide tolerance alongside functional traits to understand the adaptive and ecological potential of the bacterial isolate.

3. Results

3.1 Isolation of Bacterial Colonies

Bacterial colonies were successfully isolated from pesticide-contaminated soil using the spread plate technique on nutrient agar incorporated with monocrotophos. Growth was observed at both 100 ppm and 200 ppm concentrations. Among the serial dilutions, plates corresponding to 10^{-3} and 10^{-4} showed well-separated colonies suitable for further isolation. A decrease in colony density was observed with increasing dilution, with the 10^{-4} dilution yielding more discrete colonies.

3.2 Screening and Confirmation of Pesticide Tolerance

Colonies that developed on pesticide-containing media were selected and subjected to further screening. The selected isolate demonstrated visible growth on nutrient agar incorporated with 300 ppm monocrotophos during streak plating. This confirmed the ability of the isolate to tolerate higher concentrations of the pesticide.

3.3 Morphological and Microscopic Characteristics

The purified isolate exhibited distinct colony morphology on nutrient agar. The observed characteristics are summarized in **Table 1**.

Table 1: Colony morphology of the bacterial isolate

Parameter	Observation
Shape	Circular
Margin	Smooth
Elevation	Raised
Colour	Creamy white
Texture	Smooth
Opacity	Opaque

Microscopic examination following Gram staining revealed that the bacterial cells were Gram-negative and rod-shaped.

3.4 Biochemical Characteristics

The biochemical characteristics of the bacterial isolate are summarized in **Table 2**.

Table 2: Biochemical characteristics of the bacterial isolate

Test	Result
Indole	Negative
Methyl Red	Positive
Voges–Proskauer	Negative
Citrate utilization	Negative
Oxidase	Positive
Catalase	Positive

3.5 Plant Growth-Promoting Traits

The plant growth-promoting potential of the isolate was evaluated through selected assays. The isolate showed a positive result for ammonia production, as indicated by the development of yellow to brown coloration upon addition of Nessler's reagent (**Figure 1**).

No visible colour change was observed in the indole-3-acetic acid (IAA) assay, indicating a negative result. Similarly, no clear halo zone was observed on Pikovskaya's agar, indicating the absence of phosphate solubilization activity under the conditions tested.

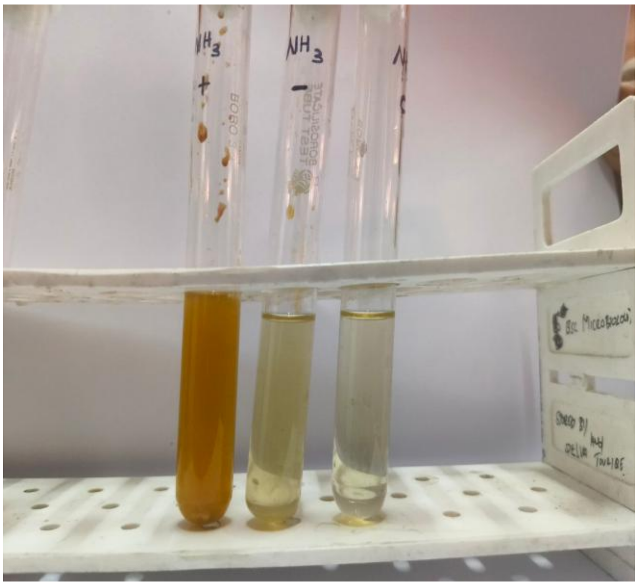

Figure 1: Ammonia production test showing development of yellow to brown coloration indicating positive result

4. Discussion

The successful isolation of bacterial colonies from pesticide-contaminated soil and their ability to grow in the presence of monocrotophos at concentrations of 100 ppm and 200 ppm indicate the presence of microbial populations capable of adapting to chemically stressed environments. The additional confirmation of growth at 300 ppm further supports the tolerance of the isolate to elevated pesticide levels. Such tolerance is commonly observed in soils subjected to repeated pesticide exposure, where selective pressure favors the survival of resistant microbial populations (Cycoń et al., 2010; Singh et al., 2004). The ability of the isolate to persist across multiple concentrations suggests a level of physiological adaptability that enables survival under adverse conditions.

The Gram-negative, rod-shaped morphology observed in the present study is consistent with characteristics commonly associated with environmentally resilient soil bacteria. Gram-negative bacteria often possess structural features, such as an outer membrane, that provide additional protection against toxic compounds, which may contribute to their survival in pesticide-contaminated environments. Although molecular identification was not performed in this study, the morphological and biochemical profile observed is in agreement with traits reported for several soil-associated bacterial genera known for stress tolerance.

Biochemical characterization of the isolate revealed a distinct metabolic profile, with positive results for methyl red, oxidase, and catalase tests, and negative results for Voges–Proskauer, citrate utilization, and indole production. The positive methyl red reaction indicates the ability of the isolate to carry out mixed-acid fermentation, which may provide metabolic flexibility under varying environmental conditions. Oxidase positivity suggests the presence of cytochrome c oxidase, indicating an active aerobic respiratory system, while catalase activity reflects the ability of the organism to neutralize reactive oxygen species generated under stress conditions. These biochemical traits collectively support the notion that the isolate is well adapted to survive in chemically stressed environments.

The evaluation of plant growth-promoting traits revealed selective functional abilities of the isolate. The positive result for ammonia production indicates the ability of the bacterium to contribute to nitrogen availability in soil through ammonification. Nitrogen is a key nutrient required for plant growth, and ammonia-producing bacteria play an important role in maintaining soil fertility, particularly under stressed conditions where microbial activity may be reduced (Bhattacharyya & Jha, 2012; Glick, 2012). The presence of this trait suggests that the isolate may contribute to basic nutrient cycling processes in pesticide-affected soils.

In contrast, the isolate did not exhibit detectable indole-3-acetic acid (IAA) production or phosphate solubilization under the conditions tested. While these traits are commonly associated with plant growth-promoting rhizobacteria, their absence does not necessarily indicate a lack of functional potential. It is well established that plant growth-promoting traits are strain-specific and may be influenced by environmental conditions, including the presence of chemical stressors (Spaepen et al., 2007). In pesticide-contaminated environments, metabolic activity may be altered, resulting in selective expression of certain traits while others remain inactive.

The coexistence of pesticide tolerance and ammonia production observed in the present study highlights the ecological significance of such bacterial isolates. Previous studies have reported that bacteria capable of surviving in pesticide-contaminated soils often retain essential metabolic functions that contribute to soil stability and nutrient cycling (Cycoń et al., 2017; Bhatt et al., 2020). This suggests that pesticide-tolerant bacteria may play a dual role in both surviving environmental stress and supporting soil functionality.

It is also important to consider the limitations of the present study. The assessment of plant growth-promoting traits was based on qualitative observations, and no quantitative measurements were performed. In addition, the study focused on a single bacterial isolate, and molecular identification was not carried out. These factors limit the ability to draw broader conclusions regarding the functional potential and taxonomic identity of the isolate. Furthermore, plant-based assays were not conducted, and therefore the direct impact of the isolate on plant growth remains to be validated.

Despite these limitations, the study provides valuable preliminary insights into the adaptive capacity of soil bacteria in pesticide-contaminated environments. The findings demonstrate that even under chemical stress, certain bacterial populations retain functional traits that may contribute to maintaining soil processes. This highlights the importance of considering both tolerance and functional potential when evaluating soil microorganisms in agricultural systems.

Overall, the results suggest that pesticide-contaminated soils harbour bacterial populations with the ability to survive chemical stress while maintaining selective functional traits. Such microorganisms may have potential applications in sustainable agriculture, particularly in improving soil fertility under conditions of pesticide exposure. However, further investigations involving molecular characterization, quantitative assays, and field-based studies are necessary to fully understand their ecological role and practical applications.

5. Conclusion

The study demonstrated the successful isolation of a pesticide-tolerant bacterial isolate from pesticide-contaminated agricultural soil, highlighting the presence of microbial populations capable of adapting to chemically stressed environments. The isolate exhibited consistent growth in the presence of monocrotophos across multiple concentrations and was characterized as a Gram-negative, rod-shaped bacterium with a defined biochemical profile. Among the plant growth-promoting traits evaluated, the isolate showed positive ammonia production, indicating its potential role in nitrogen-related processes, while indole-3-acetic acid production and phosphate solubilization were not observed under the conditions tested. This selective expression of functional traits suggests that even under pesticide stress, certain bacteria retain specific metabolic capabilities that may contribute to maintaining basic soil functions. The findings of this study provide preliminary insight into the adaptive and functional characteristics of pesticide-tolerant bacteria and emphasize their potential ecological relevance in agricultural soils exposed to chemical inputs. However, further investigations involving molecular identification, quantitative evaluation of functional traits, and plant-based studies are necessary to better understand their role and potential applications in sustainable agricultural systems.

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