

Isolation and Molecular Characterization of Monocrotophos-Tolerant *Acinetobacter baumannii* and *Pseudomonas nitroreducens* from Okra-Cultivated Soil

Dr. M. Chidambara priya¹, H.Mahmutha Rehana²

¹Assistant Professor, Department of Zoology, Vyasa Arts and Science Women's College, Subramaniapuram, Tenkasi, Tamil Nadu, India.

²III B.Sc Zoology, Vyasa Arts and Science Women's College, Subramaniapuram, Tenkasi, Tamil Nadu, India.

Abstract

Monocrotophos (MCP) is an organophosphorus pesticide widely used in agricultural practices and may persist in soil, posing potential risks to environmental and biological systems. The present study aimed to isolate and molecularly characterize bacteria capable of growing in the presence of MCP from pesticide-exposed soil collected from okra (*Abelmoschus esculentus*) cultivated fields in the Tirunelveli region of Tamil Nadu, India. Soil samples with a history of repeated organophosphate pesticide application were subjected to enrichment culture using MCP-containing mineral salt medium. Ten morphologically distinct bacterial isolates were obtained and screened for their ability to grow at increasing MCP concentrations ranging from 100 to 1000 ppm. Among the isolates, strains S7 and S9 exhibited the ability to grow at the highest tested concentration of 1000 ppm MCP. Genomic DNA was extracted from these isolates, and partial 16S rRNA gene sequences were amplified and subjected to sequence analysis. BLASTn analysis identified isolate S7 as *Acinetobacter baumannii* (GenBank accession number ON202972) and isolate S9 as *Pseudomonas nitroreducens* (GenBank accession number ON248149). Phylogenetic analysis further supported their taxonomic affiliations. The ability of these bacterial isolates to grow in the presence of high concentrations of MCP indicates their potential for application in the bioremediation of pesticide-contaminated agricultural soils. Further studies are required to quantify MCP degradation and optimize the environmental and nutritional conditions for effective biodegradation.

Keywords: Monocrotophos; organophosphorus pesticide; mineral salt medium; 16S rRNA gene; bioremediation

1. Introduction

Pesticides play an important role in modern agriculture by controlling insect pests, plant diseases, weeds, and other organisms that adversely affect crop production. Their extensive application has contributed significantly to agricultural productivity; however, excessive and repeated use of pesticides can result in the accumulation of residues in agricultural soils and may adversely affect non-target organisms and the environment. Among the different groups of pesticides, organophosphorus (OP) compounds are widely used

because of their effectiveness against a broad range of agricultural pests (Singh and Walker, 2006; Kosimov et al., 2025).

Vegetable crops are frequently exposed to pesticide applications because of their susceptibility to various insect pests. Okra (*Abelmoschus esculentus*) is an economically important vegetable crop cultivated widely in tropical and subtropical regions. Repeated application of pesticides in vegetable cultivation may result in pesticide residues in agricultural soils and can influence the microbial communities inhabiting these environments. Pesticide-contaminated agricultural soils can therefore serve as important sources for the isolation of microorganisms with pesticide tolerance and degradation potential (Rangaswamy and Venkateswarlu, 1992; Singh et al., 2003).

Monocrotophos (MCP) is a systemic organophosphorus insecticide that has been used against several agricultural insect pests. Its environmental occurrence is of concern because of its toxicity and potential movement through soil and water. A review by Sharma et al. (2021) highlighted the environmental behaviour, toxicity, and detoxification approaches associated with MCP, while Rajan et al. (2024) further emphasized the ecological concerns associated with monocrotophos exposure. Therefore, the development of effective and environmentally sustainable approaches for the remediation of MCP-contaminated environments is important.

Microbial degradation is considered an environmentally friendly approach for reducing pesticide contamination because microorganisms can transform pesticide compounds through enzymatic and metabolic processes. Microbial degradation of organophosphorus pesticides may involve specific hydrolytic enzymes and metabolic pathways that convert parent pesticide molecules into less toxic or more readily degradable compounds (Singh and Walker, 2006; Sharma et al., 2021; Kosimov et al., 2025). Native microorganisms isolated from pesticide-contaminated environments have attracted particular attention because of their adaptation to pesticide stress and their potential application in bioremediation.

Several microorganisms capable of degrading organophosphorus pesticides have been reported. Bacterial genera such as *Pseudomonas*, *Bacillus*, *Arthrobacter*, *Clavibacter*, and *Acinetobacter* have been investigated for their ability to transform or degrade different organophosphorus compounds. Previous studies have specifically reported microorganisms capable of utilizing or degrading monocrotophos, including *Pseudomonas aeruginosa*, *Clavibacter michiganense*, *Pseudomonas stutzeri*, *Pseudomonas moraviensis*, *Pseudomonas synxantha*, *Pseudomonas mendocina*, *Arthrobacter atrocyaneus*, and *Bacillus megaterium* (Lee et al., 1990; Bhadbhade et al., 2002; Singh et al., 2003; Kumar et al., 2004; Liu et al., 2009; Abraham et al., 2014; Abraham and Silambarasan, 2015). More recent investigations have also demonstrated the potential of bacterial isolates and consortia for MCP degradation. Kande et al. (2024) reported the isolation of a monocrotophos-degrading bacterial consortium from agricultural soil and demonstrated MCP degradation using chromatographic analyses. Similarly, Vaishnavi and Osborne (2024) reported that *Proteus myxofaciens* VITVJ1 exhibited resistance to MCP and other pesticides and demonstrated their degradation potential.

The isolation of pesticide-tolerant or pesticide-degrading microorganisms from contaminated environments is commonly achieved through enrichment techniques. In enrichment culture, microorganisms capable of tolerating or utilizing the target pesticide are selectively encouraged to grow in a culture medium containing

the pesticide as a selective component. This approach facilitates the recovery of microorganisms adapted to pesticide-contaminated environments and has been widely applied for the isolation of pesticide-degrading bacteria (Subhas and Singh, 2003; Buvaneswari et al., 2017; Kande et al., 2024).

Molecular characterization provides an important approach for the accurate identification of bacterial isolates. The 16S rRNA gene is widely used as a molecular marker for bacterial identification and phylogenetic analysis. Amplification and sequencing of the 16S rRNA gene, followed by comparison with sequences available in public databases using BLAST analysis, provides information on the taxonomic affiliation and phylogenetic relationships of bacterial isolates (Mullis, 1990; Castresana, 2000; Dereeper et al., 2008). Molecular identification is particularly useful when phenotypic and biochemical characteristics alone are insufficient for reliable species-level identification.

In the present study, bacterial isolates were obtained from soil collected from okra (*Abelmoschus esculentus*) cultivated fields in the Tirunelveli region of Tamil Nadu, India, where organophosphorus pesticides had been repeatedly applied. The isolates were screened for their ability to grow in mineral salt medium containing increasing concentrations of monocrotophos. The isolates showing growth at the highest tested MCP concentration were subjected to morphological, biochemical, and molecular characterization using partial 16S rRNA gene sequencing. The objective of the study was to identify MCP-tolerant bacterial isolates and evaluate their potential for application in the bioremediation of MCP-contaminated agricultural soil.

2. Materials and Methods

Chemicals

Standard analytical-grade monocrotophos (MCP) was procured from an agrochemical shop in Tirunelveli District, Tamil Nadu, India. All other chemicals and reagents used in the study were of analytical grade and were obtained from HiMedia Laboratories, India.

Collection of Soil Samples

Soil samples were collected from *Abelmoschus esculentus* (okra)-cultivated fields at different locations in and around the Tirunelveli region of Tamil Nadu, India. The selected fields had a history of repeated application of organophosphorus pesticides, including monocrotophos, based on information provided by the vegetable growers. Soil samples were collected from a depth of approximately 15 cm. The collected samples were thoroughly mixed to obtain a representative composite sample, transferred aseptically into a sterile plastic bag, and stored at 2 °C until further analysis.

Enrichment and Isolation of MCP-Tolerant Bacteria

Enrichment of soil microorganisms was carried out following the approach described by Harish et al. (2013), with modifications. Briefly, 10 g of the mixed soil sample was aseptically transferred into a sterile conical flask, and 10 mL of 100 ppm monocrotophos solution was added. The contents were mixed thoroughly using a sterile glass rod and maintained under static conditions at room temperature for five days.

Following enrichment, serial dilutions of the soil suspension were prepared using sterile distilled water. Aliquots (1 mL) from the 10^{-6} to 10^{-9} dilutions were inoculated onto mineral salt medium (MSM) plates supplemented with 100 ppm MCP using the spread-plate technique. The plates were incubated at 37 °C for 24 h.

After incubation, morphologically distinct colonies were selected and purified by repeated streaking on MSM plates containing 100 ppm MCP. The purified bacterial isolates were characterized based on their cultural and morphological characteristics and biochemical properties according to Bergey's Manual of Determinative Bacteriology (Bergey et al., 1957).

Ten morphologically distinct bacterial isolates were obtained and designated S1, S2, S3, S5, S6, S7, S9, S10, S11, and S12. The isolates were subsequently screened for their ability to grow at increasing concentrations of MCP. The isolates were sequentially transferred to MSM plates containing 250, 500, 750, and 1000 ppm MCP, with incubation at 37 °C for 24 h at each concentration. Bacterial growth was assessed based on colony development on the respective MCP-containing media. Two isolates, S7 and S9, showed growth at the highest tested concentration of 1000 ppm MCP and were selected for further characterization. The enrichment and screening strategy is consistent with approaches used for the isolation of pesticide-tolerant or pesticide-degrading microorganisms from agricultural soils. Previous studies have demonstrated the isolation of MCP-tolerant and MCP-degrading bacteria using mineral salt media containing increasing concentrations of monochrotophos (Acharya et al., 2015; Kande et al., 2024).

Genomic DNA Isolation

Genomic DNA was isolated from the selected bacterial isolates using the NucleoSpin® Tissue Kit (Macherey-Nagel, Germany) according to the manufacturer's instructions.

Agarose Gel Electrophoresis

The quality of the extracted genomic DNA was assessed by agarose gel electrophoresis. Approximately 5 µL of DNA sample was mixed with 1 µL of 6× gel-loading buffer containing 0.25% bromophenol blue and 30% sucrose in TE buffer (pH 8.0). The samples were loaded onto a 0.8% agarose gel prepared in 0.5× TBE (Tris-borate-EDTA) buffer containing ethidium bromide at a final concentration of 0.5 µg/mL.

Electrophoresis was performed in 0.5× TBE buffer at 75 V until the bromophenol blue tracking dye migrated to the bottom of the gel. The gel was visualized using a UV transilluminator (Genei), and images were captured using a Bio-Rad gel documentation system.

PCR Amplification and Purification of the 16S rRNA Gene

The partial 16S rRNA gene was amplified by polymerase chain reaction (PCR). PCR amplification was performed using the forward primer 5'-CAGGCCTAACACATGCAAGTC-3' and reverse primer 5'-GGGCGGWTGTACAAGGC-3'.

The PCR reaction mixture contained the components described in the original experimental protocol, including 5× sequencing buffer, forward and reverse primers, sequencing mix, ExoSAP-treated PCR product, Phire master mix, template DNA, and sterile distilled water. PCR amplification was performed using the following thermal cycling conditions: initial denaturation at 94 °C for 2 min, followed by 25 cycles of denaturation at 94 °C for 1 min, annealing at 52 °C for 1 min, and extension at 72 °C for 1.5 min. A final extension was performed at 72 °C for 10 min.

The amplified products were purified and subjected to sequencing. Partial 16S rRNA gene sequencing was used for the molecular identification of the selected bacterial isolates. The use of 16S rRNA gene sequencing

for bacterial identification and phylogenetic characterization is well established, although closely related bacterial species may sometimes require additional genomic or phenotypic information for definitive species-level discrimination.

BLASTn Analysis and Phylogenetic Analysis

The obtained 16S rRNA gene sequences were compared with nucleotide sequences available in the National Center for Biotechnology Information (NCBI) GenBank database using the BLASTn algorithm. The sequences showing the highest similarity were used for taxonomic identification of the bacterial isolates. Phylogenetic analysis was performed using the neighbor-joining method implemented in MEGA software. The phylogenetic relationships of the selected isolates were inferred by comparing their 16S rRNA gene sequences with closely related reference sequences available in GenBank. BLAST-based sequence comparison combined with phylogenetic analysis is a commonly used approach for bacterial taxonomic characterization.

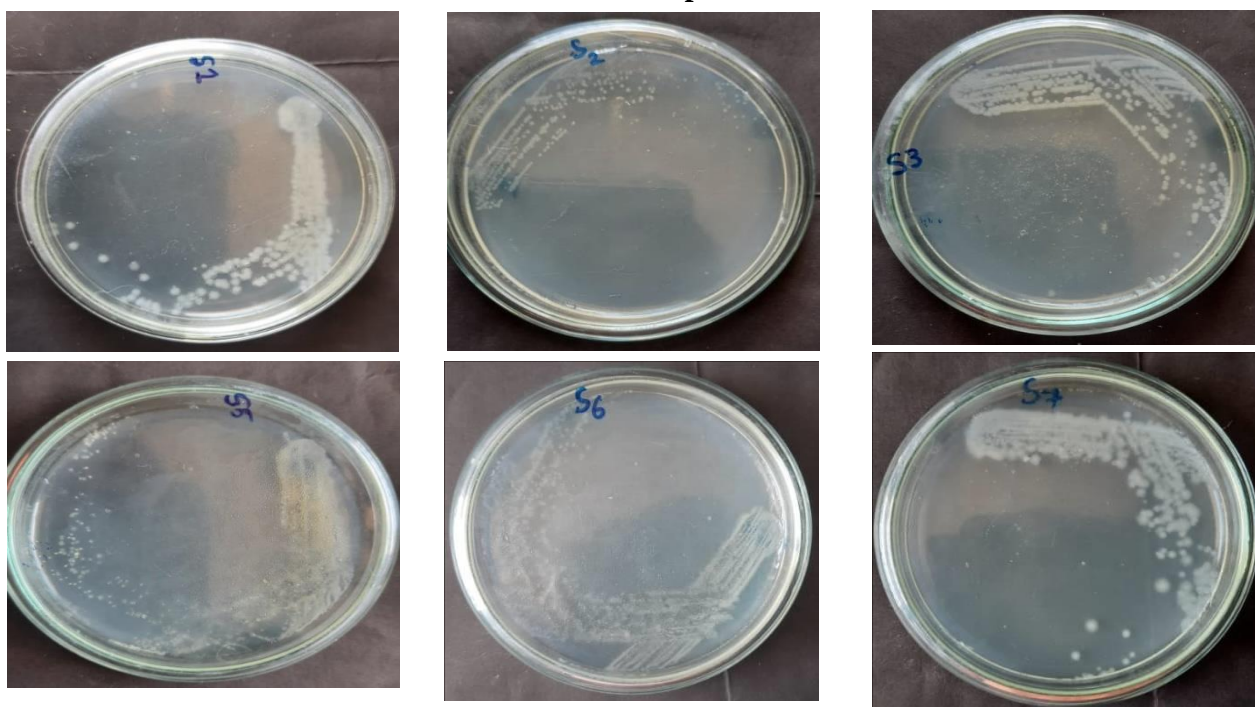
3. Results

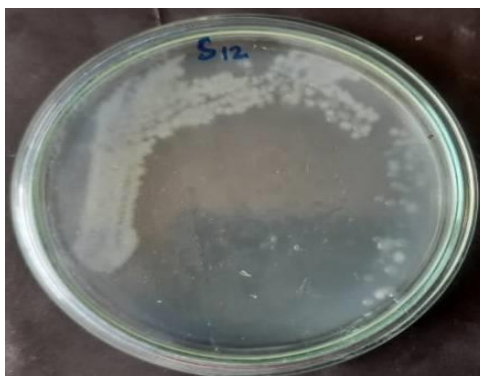
Isolation of Bacterial Strains

Bacterial isolates were obtained from soil samples collected from *Abelmoschus esculentus* cultivated fields with a history of repeated organophosphorus pesticide application. Following enrichment in monocrotophos (MCP)-supplemented mineral salt medium, serial dilution, spread plating, and repeated streaking, morphologically distinct bacterial colonies were isolated.

A total of ten morphologically distinct bacterial isolates were selected for further screening and designated S1, S2, S3, S5, S6, S7, S9, S10, S11, and S12. The purified isolates obtained on MSM supplemented with 100 ppm MCP are shown in **Figure 1**.

Figure 1. Bacterial Isolates Obtained On Mineral Salt Medium Supplemented With 1000 Ppm Monocrotophos.





Growth Response of Bacterial Isolates to Different MCP Concentrations

The ten bacterial isolates were screened for their ability to grow on mineral salt medium containing increasing concentrations of MCP (100, 250, 500, 750, and 1000 ppm). The growth response varied among the isolates (**Table 1**).

Isolates S1, S2, S5, S6, and S10 showed limited growth at increasing MCP concentrations and failed to grow at the higher concentrations tested. Isolates S3, S11, and S12 exhibited growth at some intermediate concentrations but did not grow at 1000 ppm MCP.

In contrast, isolates S7 and S9 showed comparatively higher tolerance to MCP. S7 exhibited high growth at 100 and 250 ppm and continued to grow at 1000 ppm, whereas S9 exhibited high growth up to 500 ppm and moderate growth at 750 and 1000 ppm.

Table 1. Growth Response of Bacterial Isolates At Different Concentrations Of Monocrotophos.

Isolate	100 ppm	250 ppm	500 ppm	750 ppm	1000 ppm
S1	+	+	+	—	—
S2	++	++	+	—	—
S3	+++	++	+	+	—
S5	++	++	++	—	—
S6	+++	+	+	—	—
S7	+++	+++	++	++	+
S9	+++	+++	+++	++	++
S10	++	+	+	—	—
S11	+++	+	+	+	—
S12	+++	++	++	+	—

Note: + = low growth; ++ = moderate growth; +++ = high growth; — = no visible growth.

Colony and Morphological Characteristics

The ten bacterial isolates showed variation in their colony characteristics. Four isolates were Gram-positive and six were Gram-negative. Colony pigmentation included cream, white, and yellow colours. Differences were also observed in colony size, shape, margin, and elevation.

The colonies ranged from small to large and were circular, irregular, punctiform, or rod-shaped. Colony margins were predominantly entire, undulate, or wavy, while elevations included flat, convex, and umbonate forms. The morphological characteristics of the isolates are presented in **Table 2**.

Table 2. Colony Characteristics of the Bacterial Isolates.

Isolate	Gram reaction	Size	Shape	Margin	Elevation
S1	Gram-positive	Large	Irregular	Undulated	Umbonated
S2	Gram-negative	Small	Circular	Entire	Convex
S3	Gram-negative	Small	Punctiform	Entire	Convex
S5	Gram-positive	Small	Circular	Entire	Convex
S6	Gram-positive	Small	Round	Wavy	Flat
S7	Gram-negative	Pinpoint	Irregular	Undulated	Flat
S9	Gram-negative	Small	Rod	Entire	Convex
S10	Gram-negative	Large	Circular	Entire	Flat
S11	Gram-negative	Large	Circular	Entire	Umbonated
S12	Gram-positive	Small	Punctiform	Entire	Convex

Selection of MCP-Tolerant Isolates

Among the ten isolates screened, only S7 and S9 were able to grow on MSM containing the highest tested MCP concentration of 1000 ppm. Both isolates showed clear growth in MCP-supplemented medium at 37 °C and were therefore selected for further molecular characterization.

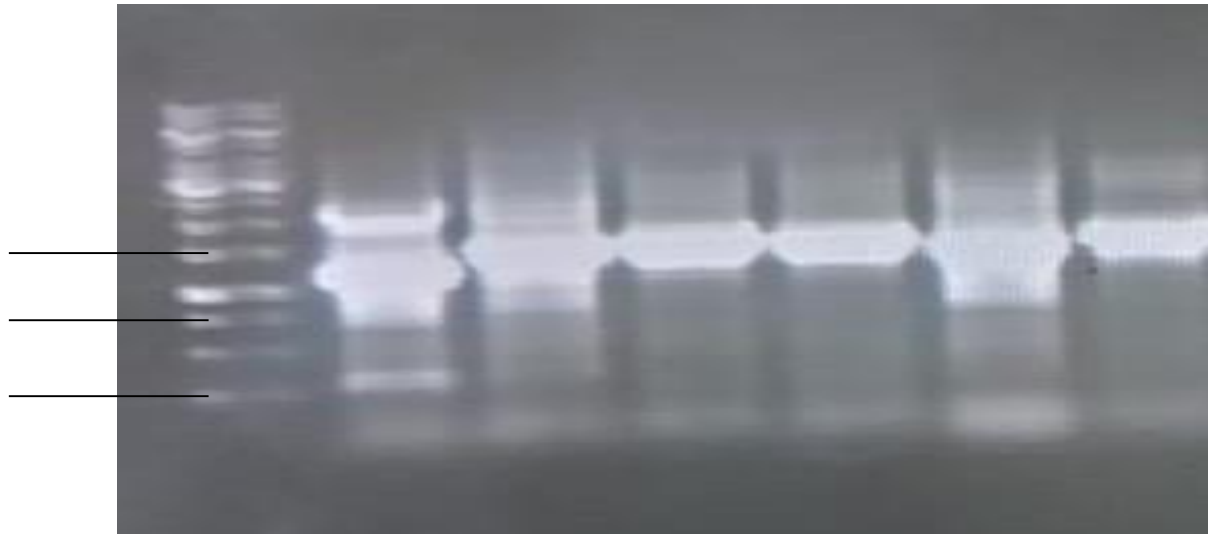
These results indicate that S7 and S9 exhibited the highest MCP tolerance among the bacterial isolates examined in the present study. However, growth in MCP-containing medium alone does not establish the rate or extent of MCP degradation.

Genomic DNA Isolation and PCR Amplification

Genomic DNA was successfully isolated from bacterial strains S7 and S9. Electrophoretic analysis using 0.8% agarose gel revealed distinct high-molecular-weight DNA bands, indicating successful genomic DNA extraction and suitable DNA quality for subsequent PCR amplification.

The partial 16S rRNA gene of both isolates was amplified using the selected forward and reverse primers. The amplified PCR products were purified prior to sequencing. The PCR products obtained from isolates S7 and S9 are shown in **Figure 2**.

Figure 2. PCR Amplification Products Of The Partial 16s Rrna Gene Of Bacterial Isolates S7 And S9.



16S rRNA Gene Sequencing

The purified PCR products were subjected to sequencing using the dideoxy sequencing method. The partial 16S rRNA gene sequence obtained for isolate S7 consisted of 735 nucleotides, whereas the sequence obtained for isolate S9 consisted of 781 nucleotides.

The obtained 16S rRNA gene sequences of S7 and S9 are presented in **Figure 3**.

Figure 3. Partial 16s Rrna Gene Sequences Obtained from Bacterial Isolates S7 and S9.

The 16S rRNA Gene Sequences of S7

```
>SR2345-S7-RSF1_E07.ab1
GGCGGACGGGTGAGTAATGCTTAGGAATCTGCCTATTAGTGGGGGACAACATCTCGAAAGGGATGCTAATAC
CGCATACG
TCCTACGGGAGAAAGCAGGGGATCTTCGGACCTTGCCTAATAGATGAGCCTAAGTCGGATTAGCTAGTTGG
TGGGGTAA
AGGCCTACCAAGGCGACGATCTGTAGCGGGTCTGAGAGGATGATCCGCCACACTGGGACTGAGACACGGCC
CAGACTCCT
ACGGGAGGCAGCAGTGGGGAATATTGGACAATGGGGGGAACCCTGATCCAGCCATGCCGCGTGTGTGAAGA
AGGCCTTAT
GGTTGTAAAGCACTTTAAGCGAGGAGGAGGCTACTCTAGTTAATACCTAGGGATAGTGGACGTTACTCGCAG
AATAAGCA
```

The 16S rRNA Gene Sequences of S9

```
>SR2345-S9-RSF1_F07.ab1
GCGGCGGACGGGTGAGTAATGCCTAGGAATCTGCCTGGTAGTGGGGGACAACGTTTCGAAAGGAACGTAATACGCATA
CGTCCTACGGGAGAAAGCAGGGGACCTTCGGGCCTTGCCTATCAGATGAGCCTAGGTCGGATTAGCTAGTTGGTGGG
GT
AAAGGCCTACCAAGGCGACGATCCGTAACCTGGTCTGAGAGGATGATCAGTCACACTGGAACCTGAGACACGGTCCAGACT
C CTACGGGAGGCAGCAGTGGGGAATATTGGACAATGGGCGAAAGCCTGATCCAGCCATGCCGCGTGTGTGAAGAAGGTCTT
CGGATTGTAAAGCACTTTAAGTTGGGAGGAAGGGCAGTAAGTTAATACCTTGCTGTTTTGACGTTACCAACAGAATAAG
C
ACCGGCTAACTTCGTGCCAGCAGCCGCGGTAATACGAAGGGTGCAAGCGTTAATCGGAATTACTGGGCGTAAAGCGCG
CG
TAGGTGGTTTGGTAAGATGGATGTGAAATCCCCGGGCTCAACCTGGGAACTGCATCCATAACTGCCTGACTAGAGTACG
G TAGAGGGTGGTGAATTCCTGTGTAGCGGTGAAATGCGTAGATATAGGAAGGAACACCAGTGGCGAAGGCGACCACCTG
GACTGATACTGACACTGAGGTGCGAAAGCGTGGGAGCAAACAGGATTAGATACCTGGTAGTCCACGCCGTAAACGATG
TCGACTAGCCGTTGGGATCCTTGAGATCTTAGTGGCGCAGCTAACGCGATAAGTCGACCGC
```

Molecular Identification by BLASTn Analysis

The obtained partial 16S rRNA gene sequences were subjected to BLASTn analysis against nucleotide sequences available in the NCBI GenBank database.

Based on sequence similarity, isolate S7 was identified as *Acinetobacter baumannii*, with GenBank accession number **ON202972**, whereas isolate S9 was identified as *Pseudomonas nitroreducens*, with GenBank accession number **ON248149**.

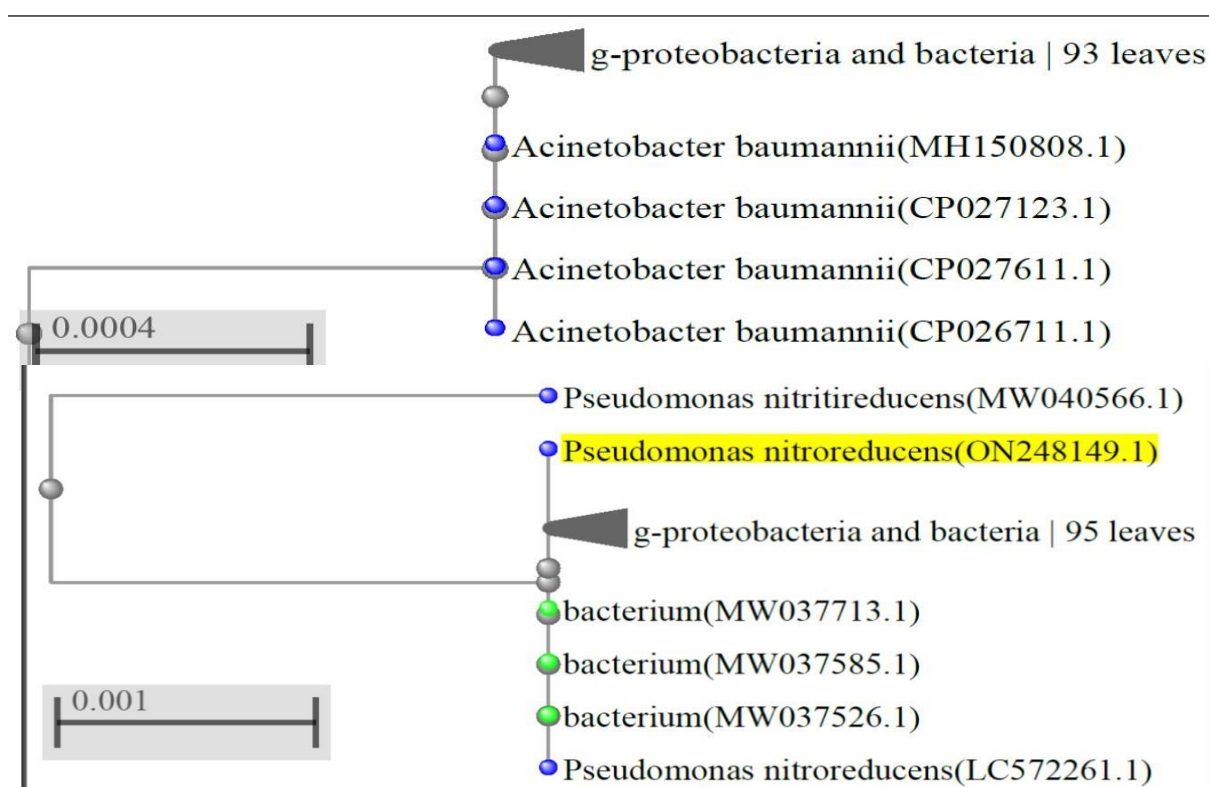
The molecular identification was further supported by phylogenetic analysis based on comparison with closely related bacterial sequences.

Phylogenetic Analysis

A phylogenetic tree was constructed using the neighbor-joining method to determine the phylogenetic relationships of isolates S7 and S9 with closely related bacterial taxa.

The phylogenetic analysis showed that isolate S7 clustered with *Acinetobacter baumannii*, while isolate S9 clustered with *Pseudomonas nitroreducens*. The phylogenetic relationships of the isolates are presented in **Figure 4**.

Figure 4. Phylogenetic Analysis of *Acinetobacter Baumannii* S7 and *Pseudomonas Nitroreducens* S9 Based On Partial 16s Rrna Gene Sequences.



4. Summary of Molecular Identification

The results of morphological characterization, MCP tolerance screening, 16S rRNA gene sequencing, BLASTn analysis, and phylogenetic analysis identified the two selected MCP-tolerant isolates as *Acinetobacter baumannii* S7 (ON202972) and *Pseudomonas nitroreducens* S9 (ON248149).

The present results demonstrate that these isolates were capable of growing in mineral salt medium containing MCP concentrations up to 1000 ppm. Their ability to tolerate high MCP concentrations suggests their potential for further investigation in MCP biodegradation and bioremediation studies.

5. Discussion

The present study focused on the isolation and molecular characterization of bacterial isolates obtained from monocrotophos (MCP)-exposed soil associated with okra (*Abelmoschus esculentus*) cultivation in the Tirunelveli region of Tamil Nadu, India. Enrichment using MCP-containing mineral salt medium enabled the recovery of bacterial isolates capable of growing under pesticide-selective conditions. Ten morphologically distinct isolates were subsequently screened at increasing MCP concentrations, of which isolates S7 and S9 retained visible growth at the highest tested concentration of 1000 ppm MCP.

The occurrence of pesticide-tolerant microorganisms in pesticide-exposed agricultural soils is expected because repeated exposure to pesticides can exert selective pressure on indigenous microbial populations. Such environments can therefore provide a useful source for the isolation of microorganisms with potential for pesticide transformation and bioremediation. Previous investigations have reported the isolation of MCP-degrading bacteria from agricultural soils, including *Arthrobacter atrocyaneus*, *Bacillus megaterium*, *Bacillus subtilis*, *Starkeya novella*, and *Sphingobium* sp.

The ability of S7 and S9 to grow in medium containing up to 1000 ppm MCP is noteworthy. A comparable concentration has been reported in previous MCP biodegradation studies. Acharya et al. (2015) reported that *Bacillus subtilis* KPA-1 was able to grow in minimal salt medium containing 1000 ppm MCP and achieved 94.2% degradation under optimized aerobic conditions. However, the present study did not quantify MCP disappearance and therefore the growth observed for S7 and S9 should be interpreted as evidence of high MCP tolerance rather than direct evidence of a quantified degradation rate.

Previous studies have demonstrated that MCP can be biologically transformed by diverse bacterial taxa. Bhadbhade et al. reported MCP mineralization by *Arthrobacter atrocyaneus* and *Bacillus megaterium*, with degradation of MCP accompanied by formation of metabolites and detection of phosphatase and esterase activities. Similarly, *Starkeya novella* YW6 was reported to utilize MCP as a carbon and nitrogen source and completely degrade 0.2 mM MCP within 36 h under the reported experimental conditions. These findings indicate that different bacterial species may employ different enzymatic pathways for MCP transformation.

More recent studies further support the potential of indigenous bacteria for MCP bioremediation. Kande et al. (2024) isolated a bacterial consortium from agricultural soil and demonstrated MCP degradation using HPLC and GC-MS analyses. Their study showed complete degradation of MCP in soil after incubation with the bacterial consortium, with the formation of less toxic products. Similarly, Vaishnavi and Osborne (2024) reported that *Proteus myxofaciens* VITVJ1 isolated from agricultural soil exhibited resistance to MCP and demonstrated degradation of MCP and other pesticides.

The molecular identification of S7 and S9 provided further insight into the taxonomic identity of the selected isolates. Based on partial 16S rRNA gene sequence analysis, isolate S7 was identified as *Acinetobacter baumannii* (ON202972), while S9 was identified as *Pseudomonas nitroreducens* (ON248149). The BLASTn results, together with the phylogenetic analysis, supported the taxonomic affiliations of the two isolates. Molecular identification based on 16S rRNA gene sequencing is widely used for bacterial characterization; however, closely related bacterial taxa may require additional molecular markers or whole-genome approaches for definitive species-level resolution.

The identification of *Acinetobacter baumannii* and *Pseudomonas nitroreducens* in the present study is of interest because members of both genera have been reported in environmental and pesticide-associated microbial studies. However, the present results should not be interpreted as establishing that these species are universally capable of degrading MCP. Rather, the findings demonstrate that the isolates obtained in this study were able to grow under the tested MCP-selective conditions. Their potential MCP-degrading activity requires confirmation through direct measurement of MCP disappearance and identification of degradation products.

This distinction is important because previous studies that established MCP biodegradation generally used analytical techniques such as HPLC, GC-MS, or metabolite analysis. For example, Kande et al. (2024) confirmed MCP degradation using HPLC and GC-MS, while recent work published in 2026 reported 88–90% MCP biodegradation by *Stenotrophomonas pavanii* and *Brevibacillus parabrevis* using chromatographic and spectroscopic approaches.

The high MCP tolerance observed for S7 and S9 therefore provides a useful basis for further investigation. Future studies should determine the actual MCP degradation efficiency of these isolates under controlled conditions, including different MCP concentrations, incubation periods, pH, temperature, carbon and nitrogen sources, and inoculum concentrations. Quantification of MCP by HPLC or GC-MS would provide direct evidence of degradation, while analysis of degradation metabolites could help establish the metabolic pathway involved. Screening for genes such as *opd*, *opdA*, or other organophosphate-hydrolase-associated genes could additionally provide information on the molecular mechanisms responsible for MCP transformation. Previous MCP-degrading bacteria have been shown to harbour organophosphate hydrolase genes and associated enzymatic activities.

Overall, the present study demonstrates the successful isolation and molecular identification of two bacterial isolates, *Acinetobacter baumannii* S7 and *Pseudomonas nitroreducens* S9, capable of growing in mineral salt medium containing MCP up to 1000 ppm. The findings suggest that these isolates are promising candidates for further evaluation as potential agents for the bioremediation of MCP-contaminated agricultural soils. Nevertheless, direct MCP degradation assays are required before their biodegradation efficiency can be conclusively established.

6. Conclusion

The present study successfully isolated and molecularly characterized bacterial strains from monocrotophos-exposed soil associated with okra (*Abelmoschus esculentus*) cultivation in the Tirunelveli region of Tamil Nadu, India. Among the bacterial isolates obtained through enrichment and selective screening, isolates S7 and S9 showed the highest tolerance to monocrotophos and were able to grow in mineral salt medium containing up to 1000 ppm monocrotophos.

Based on partial 16S rRNA gene sequence analysis and BLASTn comparison, isolate S7 was identified as *Acinetobacter baumannii* (ON202972), while isolate S9 was identified as *Pseudomonas nitroreducens* (ON248149). Phylogenetic analysis further supported the taxonomic affiliation of these isolates.

The ability of these isolates to grow under high monocrotophos concentrations indicates their potential as candidates for further investigation in pesticide bioremediation. However, the present study demonstrates monocrotophos tolerance rather than quantitatively confirmed pesticide degradation. Therefore, further

studies involving HPLC or GC-MS-based estimation of monocrotophos removal, identification of degradation metabolites, enzyme assays, and screening for organophosphate-degrading genes such as *opd* and *opdA* are required to confirm their biodegradation capability.

Overall, the isolates *A. baumannii* S7 and *P. nitroreducens* S9 represent promising bacterial candidates for future studies on the biological transformation and possible bioremediation of monocrotophos-contaminated agricultural soils.

References

1. Acharya, K.P., Shilpkar, P., Shah, M.C. and Chellapandi, P. (2015). Biodegradation of insecticide monocrotophos by *Bacillus subtilis* KPA-1, isolated from agriculture soils. *Applied Biochemistry and Biotechnology*, 175(4), 1789–1804. <https://doi.org/10.1007/s12010-014-1401-5>
2. Bhadbhade, B.J., Sarnaik, S.S. and Kanekar, P.P. (2002). Biomineralization of an organophosphorus pesticide, monocrotophos, by soil bacteria. *Journal of Applied Microbiology*, 93(2), 224–234. <https://doi.org/10.1046/j.1365-2672.2002.01680.x>
3. Bhadbhade, B.J., Sarnaik, S.S. and Kanekar, P.P. (2002). Bioremediation of an industrial effluent containing monocrotophos. *Current Microbiology*, 45(5), 346–349. <https://doi.org/10.1007/s00284-002-3681-1>
4. Castresana, J. (2000). Selection of conserved blocks from multiple alignments for their use in phylogenetic analysis. *Molecular Biology and Evolution*, 17(4), 540–552. <https://doi.org/10.1093/oxfordjournals.molbev.a026334>
5. Dereeper, A., Guignon, V., Blanc, G., Audic, S., Buffet, S., Chevenet, F., Dufayard, J.-F., Guindon, S., Lefort, V., Lescot, M., Claverie, J.-M. and Gascuel, O. (2008). Phylogeny.fr: robust phylogenetic analysis for the non-specialist. *Nucleic Acids Research*, 36(Suppl. 2), W465–W469. <https://doi.org/10.1093/nar/gkn180>
6. Kande, R., Rajkumar, K., Anoor, P.K., Naik, S. and Burgula, S. (2024). Isolation of monocrotophos degrading bacterial consortium from agricultural soil for in vivo analysis of pesticide degradation. *Brazilian Journal of Microbiology*, 55(4), 4101–4114. <https://doi.org/10.1007/s42770-024-01497-6>
7. Kosimov, D., Ergashev, R., Mavjudova, A. and Kuziev, S. (2025). Organophosphorus pesticide degradation by microorganisms: A review. *Frontiers in Bioscience (Elite Edition)*, 17(3), Article 38805. <https://doi.org/10.31083/FBE38805>
8. Rangaswamy, V. and Venkateswarlu, K. (1992). Degradation of selected insecticides by bacteria isolated from soil. *Bulletin of Environmental Contamination and Toxicology*, 49(6), 797–804. <https://doi.org/10.1007/BF00203150>
9. Singh, B.K. and Walker, A. (2006). Microbial degradation of organophosphorus compounds. *FEMS Microbiology Reviews*, 30(3), 428–471. <https://doi.org/10.1111/j.1574-6976.2006.00018.x>
10. Vaishnavi, J. and Osborne, J.W. (2024). Biodegradation of monocrotophos, cypermethrin and fipronil by *Proteus myxofaciens* VITVJ1: A plant–microbe-based remediation. *Heliyon*, 10(18), e37384. <https://doi.org/10.1016/j.heliyon.2024.e37384>