Biodegradation of Paraquat and Profenofos by Exiguobacterium profundum: Harnessing Enzymatic, Phytotoxicity, and Plant Growth-Promoting Properties

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Abstract:

Pesticide contamination of soils and water environments is a serious ecological problem, and it clearly calls for remediation methods that are both efficient and sustainable. Paraquat, a highly toxic bipyridyl herbicide, and profenofos, a broad-spectrum organophosphorus insecticide, are among the most widely used pesticides, yet they remain long-lasting in the environment with little ease of breakdown. Microbial bioremediation stands out as a practical alternative because it is cost-effective, eco-friendly, and capable of detoxifying contaminated sites without generating secondary pollutants. In this study, we examined the biodegradation potential of Exiguobacterium profundum (isolate MAK1), a polyextremophilic Gram-positive bacterium, against paraquat and profenofos under laboratory conditions. Identification by 16S rRNA gene sequencing showed 100% identity (911/911 nucleotides) with Exiguobacterium profundum strain 0056 (GenBank: KP236196.1). Tolerance assays showed a concentration-dependent decline in growth, although the isolate still grew strongly at 50 ppm and moderately at 100 ppm for both pesticides. Over 10 days in Bushnell–Haas broth, paraquat degradation rose from about 31% on day 0 to 92.32% on day 10 at 50 ppm, while at 100 ppm it increased from 25% to 80.37%. Profenofos degradation followed a similar pattern, progressed from 32% to 78.4% at 50 ppm, whereas at 100 ppm it increased only from about 1.2% to 27.03%. Esterase activity, tested on tributyrin agar, supported an enzymatic role in pesticide breakdown, especially in the case of profenofos. Phytotoxicity assays using Vigna radiata (mung bean) seeds demonstrated that microbial treatment greatly reduced toxicity, with treated paraquat and profenofos samples producing much higher seedling vigor indices (694.4 and 535.46, respectively) than untreated samples (339.5 and 211.10). Inhibition tests against Staphylococcus aureus and Escherichia coli also showed that the degraded samples were less toxic. The isolate also displayed useful plant growth-promoting traits, including indole-3-acetic acid (IAA) production, siderophore synthesis, and ammonia production. These findings presents E. profundum as a promising multifunction bioremediation and plant growth-promoting organism for managing pesticide-contaminated agricultural environments.

1. Introduction:

Pesticides remain essential to modern agriculture because they protect crops from insects, weeds, fungi, and other pests that reduce yield. According to the Food and Agriculture Organization (FAO), global agricultural pesticide use reached about 3.73 million tonnes of active ingredients in 2023, nearly twice the level recorded in 1990, with an average application rate of 2.40 kg per hectare of cropland (FAO, 2025). Even so, only a small portion of what is applied actually reaches the target pest. The rest ends up in soil, water, and air, where it

contaminates agricultural ecosystems and imposes risk to beneficial organisms and human health (Tudi et al., 2021; Kaur et al., 2024).

Paraquat (1,1′-dimethyl-4,4′-bipyridinium dichloride) is a non-selective, fast-acting contact herbicide classified as a bipyridyl compound. It is highly soluble in water, yet it adheres strongly to clay minerals and organic matter, which gives it soil half-lives that can stretch from months to years. Paraquat produces reactive oxygen species by diverting electrons from photosystem I, leading to rapid plant necrosis and, in mammals, severe pulmonary fibrosis and oxidative injury (Huang et al., 2019; Inthama et al., 2021). Profenofos [O-(4-bromo-2-chlorophenyl) O-ethyl S-propyl phosphorothioate] is a broad-spectrum organophosphorus insecticide and acaricide widely applied to cotton, vegetables, and fruit crops. It inhibits acetylcholinesterase, which causes neurotoxicity in target and non-target organisms alike, and it is classified by WHO as toxicity class II, meaning moderately hazardous (Kushwaha et al., 2016; Raj et al., 2024).

Physico-chemical remediation methods such as advanced oxidation, adsorption, incineration, membrane filtration, electrochemical treatment, and chemical precipitation are often expensive to run, energy-demanding, and capable of generating secondary pollutants (Bose et al., 2021; Raffa & Chiampo, 2021). Microbial biodegradation offers a different strategies for this harmful pesticides. It relies on the metabolic activities of bacteria, fungi, and algae to transform harmful pesticide molecules into less toxic products (Bhatt et al., 2021; Kumar et al., 2018). Among pesticide-degrading bacteria, Exiguobacterium has drawn increasing attention because of its polyextremophilic nature, metabolic flexibility, and known ability to break down different environmental pollutants (Kasana & Pandey, 2018; Xiao et al., 2024).

Exiguobacterium profundum was first described by Crapart et al. (2007) from a deep-sea hydrothermal vent at the East Pacific Rise, where it was identified as a moderately thermophilic, halotolerant, facultatively anaerobic, non-spore-forming Gram-positive bacterium. Later studies recovered members of this species from mine soil, petroleum-contaminated sites, saline sediments, arsenic-rich environments, and Tibetan glaciers, which shows its versatile adaptability (Kasana & Pandey, 2018; Zhang et al., Das et al. 2021, Delegan et al. 2021, Barghoth et al. 2023, Sengupta et al. 2020, Tedesco et al. 2021). Sur & Sathiavelu (2024) reported that an Exiguobacterium sp. from rhizospheric soil degraded the organophosphate dimethoate with approximately 95.87% efficiency at 150 ppm within five days. Isworo & Sabdono (2016) showed profenofos degradation by a consortium of Exiguobacterium profundum and Oceanobacillus iheyensis within 192 hours. After this much

studies, the degradation of paraquat by E. profundum, along with enzyme involvement, detoxification, and PGP traits in one experimental setup, remains largely unexplored.

The present study aimed to characterise the bacterial isolate MAK1 through morphological and molecular (16S rRNA) methods, evaluate its tolerance and degradation efficiency toward paraquat and profenofos at two concentrations (50 and 100 ppm) over 10 days, assess esterase enzyme involvement, verify detoxification through phytotoxicity assays using Vigna radiata and microbial inhibition tests, and identify plant growth-promoting traits such as IAA production, siderophore production, and ammonia production.

2. Materials and Methods

2.1 Bacterial Strain and Culture Maintenance

The bacterial strain Exiguobacterium profundum (isolate MAK1) was obtained from the Department of Biosciences, VNSGU, Surat. Pure cultures were maintained on Nutrient Agar (NA) by streak plate technique, incubated at 30–37°C for 24–48 h, and stored on Nutrient agar slants at 4°C for further experimental use.

2.2 Pesticides and Media

Paraquat dichloride and profenofos (technical grade) were used as target pesticides. Stock solutions were prepared in sterile distilled water (paraquat) or dimethyl sulfoxide—DMSO (profenofos) and were filter-sterilized (0.22 µm). Bushnell–Haas (BH) media was used as the minimal media for tolerance and degradation tests.

2.3 Morphological and Molecular Characterisation

Morphological characterisation was performed by observing colony characteristics on Nutrient Agar after incubation of 24 hrs at 30°C, including shape, size, margin, elevation, opacity, texture, and pigmentation. Gram staining was performed and cells were observed under 100× oil immersion lens. For molecular identification, the 16S rRNA gene (911 bp, isolate MAK1) was sequenced and subjected to BLASTn analysis against the NCBI core nucleotide database. Sequence identity, query coverage, E-value, and bit score were evaluated.

2.4 Tolerance Assay

Bushnell–Haas agar supplemented with paraquat or profenofos at 50 ppm and 100 ppm concentrations was prepared aseptically (Jokhakar et al., 2022). The bacterial culture was spot-inoculated at the center of each plate and incubated at 30–37°C for 24–72 hrs. Plates were examined for colony growth and formation of clear zones, which would indicate utilization or degradation of the pesticide.

2.5 Pesticide Degradation in Liquid Medium

Bushnell–Haas broth was supplemented with either paraquat or profenofos at 50 ppm and 100 ppm (Jokhakar et al., 2022). Actively growing cultures of Exiguobacterium profundum

(1–2 mL) were inoculated into 100 mL pesticide-supplemented broth. Uninoculated flasks with identical pesticide concentrations served as controls. Flasks were incubated at 30–37°C at 120–150 rpm for 10 days. Samples were collected at intervals of 0, 24, 48, and 72 hrs, and subsequently every 24 hrs up to day 10. Absorbance was measured by UV-Visible spectrophotometry at the respective wavelength of each pesticide. Standard calibration curves were constructed to convert OD values to pesticide concentration. Percentage degradation was calculated as shown below:

% Degradation = [(OD control – OD sample) / OD control] × 100

2.6 Esterase Enzyme Activity Assay

Esterase activity was assessed both qualitatively and semi-quantitatively using Tributyrin Agar (Jokhakar et al., 2022). Mineral Salt Medium (MSM) agar plates supplemented with 500 ppm monocrotophos and 1% tributyrin were additionally prepared. Bacterial cultures (5 µL) were spot-inoculated at the center of each plate and incubated at 37°C for 48 hrs. Hydrolysis of tributyrin, producing a clear halo zone around the colony, indicated esterase activity. The Solubilization Index (SI) was calculated as:

SI = (Diameter of colony (mm) + Diameter of halo zone (mm)) / Diameter of colony (mm)

2.7 Phytotoxicity Assay

The phytotoxicity of untreated and microbially degraded pesticide samples was evaluated using a seed germination bioassay with Vigna radiata (mung bean) as the test plant (Jyoti et al., 2024; Abdul-Baki & Anderson, 1973). Seeds were surface-sterilised by immersing in 0.1% $HgCl_2$ for 1–2 min, washed 4–5 times with sterile distilled water, and air-dried. Thirty seeds were placed on Whatman No. 1 filter paper in sterile Petri dishes (90 mm) and respective test solution was added to moisten the filter paper. Three treatment conditions were tested in triplicate: (1) sterile distilled water (control); (2) treated paraquat or profenofos (degraded supernatant); (3) untreated paraquat or profenofos. Plates were incubated in the dark at 25 ± 2°C for 5–7 days. Moisture was maintained by adding small volumes of the respective test solutions when necessary. Germination percentage, mean shoot length, mean root length, total seedling length, and Seedling Vigour Index (SVI-I) were calculated using:

SVI-I = Germination% × (Mean Root Length + Mean Shoot Length)

- Germination Percentage (%) = (Number of germinated seeds / Total seeds) × 100
    - Mean Root Length (cm) = average root length of seedlings
    - Mean Shoot Length (cm) = average shoot length of seedlings

2.8 Microbial Inhibition Test

Antimicrobial activity of untreated and degraded pesticide samples was tested against Staphylococcus aureus and Escherichia coli by the agar well diffusion method (Aftab et al., 2011). Sterile Nutrient Agar plates were prepared and evenly inoculated with the microbial culture using a sterile cotton swab to form a uniform lawn. Wells (6 mm diameter) were punched with a sterile cork borer, and 50–100 µL of each test sample was loaded. Plates were incubated at 30–37°C for 24 hrs. The diameter of inhibition zones (mm) was measured.

2.9 Plant Growth-Promoting (PGP) Trait Assessment

2.9.1 *Indole-3-Acetic Acid (IAA) Production*

The indole acetic acid (IAA) production was measured using a 5 mL supernatant (with and without Pesticide). The supernatant was mixed with 0.5 mL of orthophosphoric acid and 5 mL of Salkowaski's reagent. The development of pink color indicates the IAA production, which was quantified at 530 nm . (Rodrigues et al., 2016)

2.9.2 *Siderophore Production*

Siderophore production was assessed on Chrome Azurol S (CAS) agar. Isolates were spot-inoculated at the center of CAS plates (with and without pesticide supplementation) and incubated for 4–5 days. Formation of orange/pink halos around colonies against the blue-green CAS background was considered positive (Jokhakar et al., 2022)

2.9.3 *Ammonia Production*

The isolate was inoculated in peptone water and incubated for 96 hrs. Ammonia production was confirmed by adding Nessler's reagent drops to the supernatant; brown colour development indicated a positive result (Nagalingam et al., 2020).

3. Results

3.1 Morphological Characterisation

The isolate MAK1 produced circular, moderate-sized, convex, opaque, smooth, and bright yellow-pigmented colonies on Nutrient Agar after 24 hrs incubation at 30°C (Table 1). Gram staining showed gram-positive rod-shaped cells which are characteristic features of Exiguobacterium profundum (Crapart et al., 2007; Kasana & Pandey, 2018).

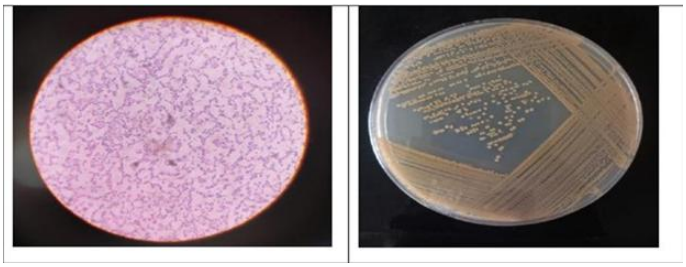


Figure 1. (Left) Gram-stained cells of Exiguobacterium profundum MAK1 showing Gram-positive rods (100× oil immersion). (Right) Colony morphology on Nutrient Agar after 48 h incubation showing yellow pigmented colonies.

Table 1. Morphological characteristics of isolate MAK1 on Nutrient Agar

Feature	Observation
Colony Shape	Circular
Size	Moderate
Margin	Entire
Elevation	Convex
Opacity	Opaque
Texture	Smooth
Colour	Yellow
Gram Reaction	Gram-positive rods

3.2 Molecular Identification: 16S rRNA Gene Sequencing and BLAST Analysis

As shown in the results (Figure 2), The BLASTn results (RID: WJ3MREV2014) revealed that the isolate MAK1 belongs to the genus Exiguobacterium. The sequence showed a 100% identity ($911/911$ nucleotides) with the reference sequence of Exiguobacterium profundum strain 0056 (Accession Number: KP236196.1). The alignment yielded a significant E-value of 0.0, a total score of 1683 bits, and 0% gaps, indicating a perfect match. Based on these high-stringency parameters, the isolate MAK1 is conclusively identified as Exiguobacterium. (Figure 2)

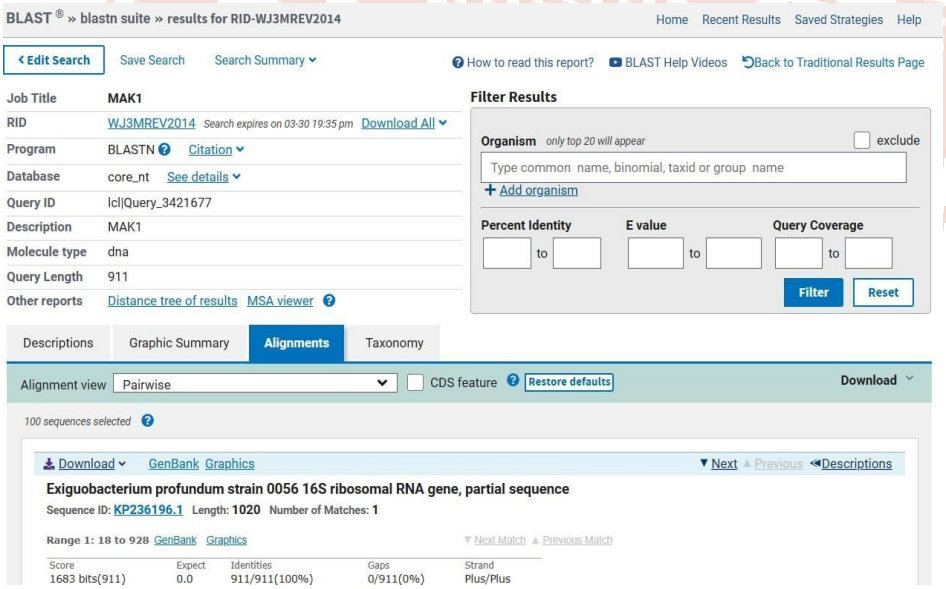
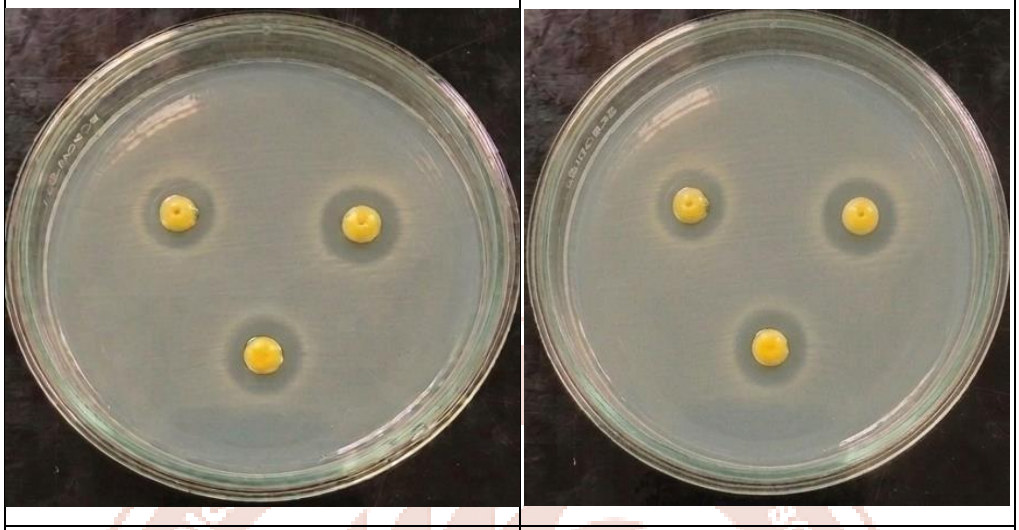


Figure 2. BLASTn results showing 100% sequence similarity of isolate MAK1 with Exiguobacterium profundum strain 0056 (KP236196.1), confirming species-level identification.

3.3 Tolerance Assay

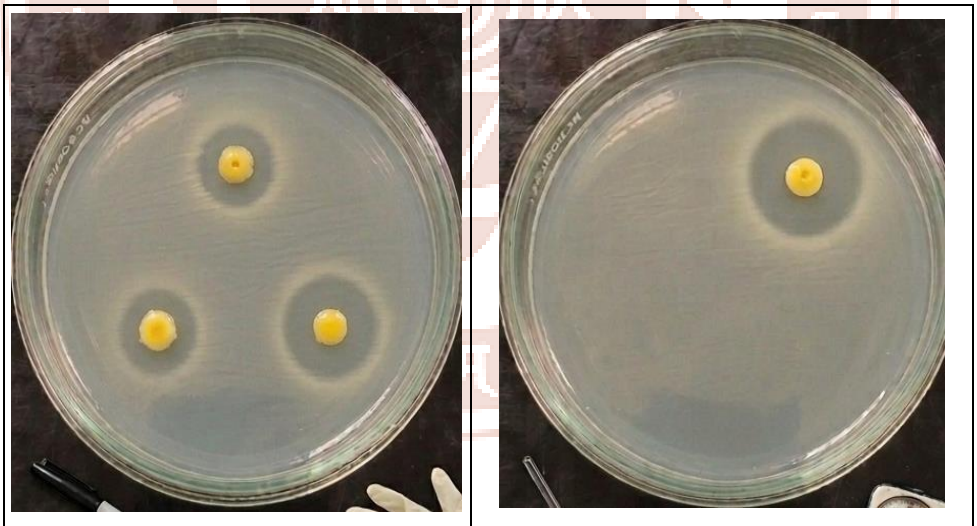
Exiguobacterium profundum MAK1 showed growth on BH agar supplemented with both pesticides, confirming its ability to tolerate paraquat and profenofos. Higher growth was

observed at 50 ppm concentrations of both paraquat and profenofos, while a significant reduction occurred at 100 ppm, indicating concentration-dependent inhibition (Figure 3 and 4).



***Exiguobacterium profundum* on BH media supplemented with 50 ppm paraquat**	***Exiguobacterium profundum* on BH media supplemented with 100 ppm paraquat**

Figure 3. Tolerance of Exiguobacterium profundum MAK1 on BH agar supplemented with paraquat at 50 ppm (left) and 100 ppm (right), showing concentration-dependent growth reduction.



***Exiguobacterium profundum* on BH media supplemented with 50 ppm profenofos**	***Exiguobacterium profundum* on BH media supplemented with 100 ppm profonofos**

Figure 4. Tolerance of Exiguobacterium profundum MAK1 on BH agar supplemented with profenofos at 50 ppm (left) and 100 ppm (right).

3.4 Percentage Degradation of Pesticides

3.4.1 *Paraquat Degradation*

A standard calibration curve for paraquat exhibited a strong linear relationship (R^2 = 0.9469) between concentration (50–1000 mg/L) and optical density. Paraquat degradation by E. profundum MAK1 increased progressively over 10 days at both concentrations tested (Figure 5 and 6). At 50 ppm, degradation gradually rose from 31% on day 0 to 92.32% on day 10. At 100 ppm, degradation progressed from 25% on day 1 to 80.37% on day 10, demonstrating efficient biodegradation at both concentrations.

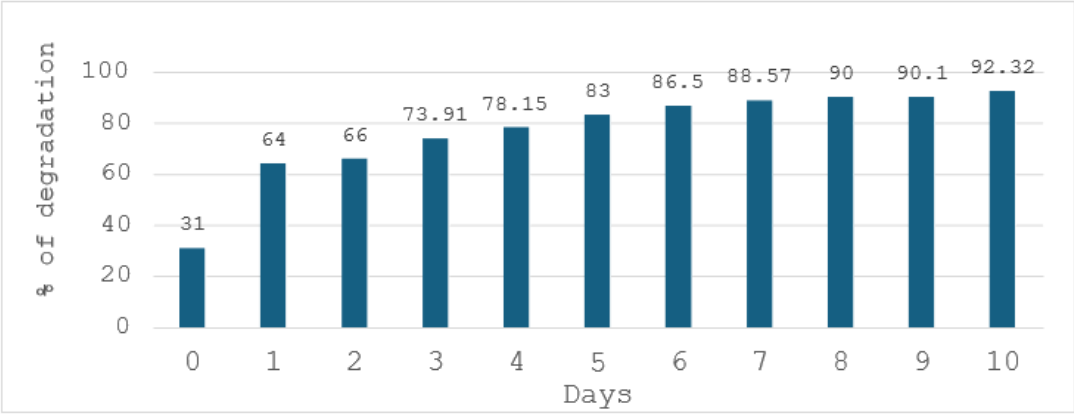


Figure 5. Percentage degradation of paraquat at 50 ppm concentration by Exiguobacterium profundum MAK1 over 10 days.

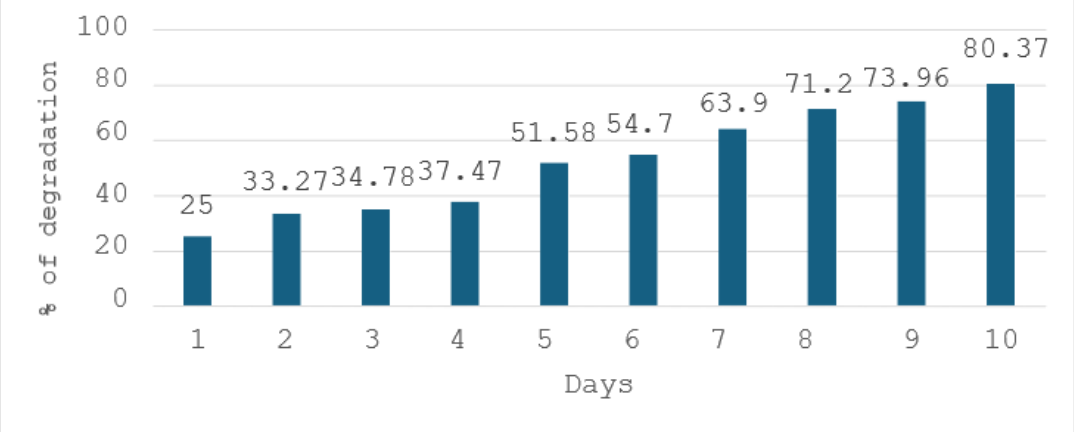


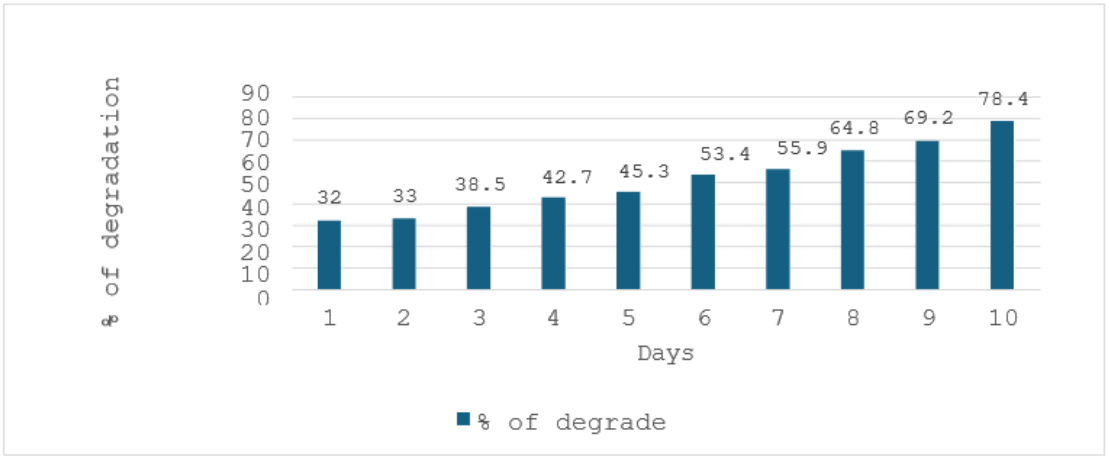
Figure 6. Percentage degradation of paraquat at 100 ppm concentration by Exiguobacterium profundum MAK1 over 10 days.

Table 2. Percentage degradation of paraquat by *Exiguobacterium profundum* MAK1 over 10 days

Concentration	Day 1	Day 3	Day 5	Day 7	Day 10
50 ppm	64%	73.91%	83%	88.57%	92.32%
100 ppm	25%	34.78%	51.58%	63.9%	80.37%

3.4.2 *Profenofos Degradation*

The profenofos standard calibration curve also showed a reliable linear relationship ($R^2 = 0.9291$). Profenofos degradation was lower overall compared to paraquat (Figure 7). At 50 ppm, degradation increased from 32% on day 1 to 78.4% on day 10. At 100 ppm, a significantly lower degradation rates were observed, rising from ~1.2% on day 1 to 27.03% on day 10. This suggests that degradation works better at lower concentration while compared at higher concentration where it's inhibited due to substrate toxicity.



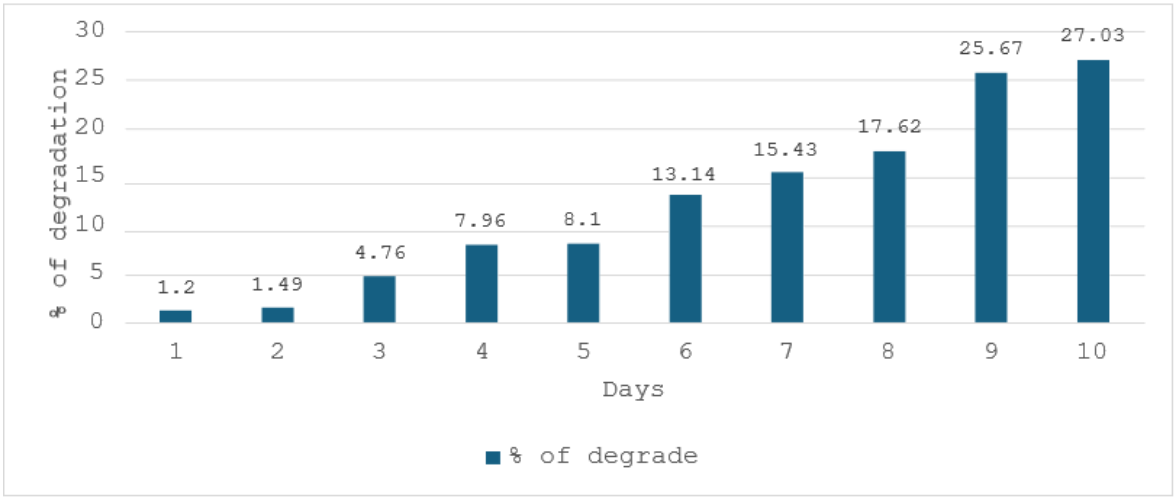


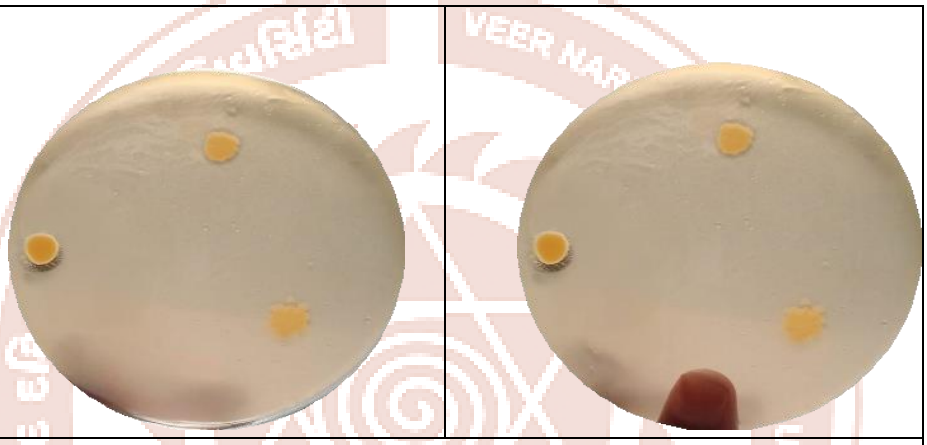
Figure 7 & 8. Percentage degradation of profenofos at 50 ppm (top) and 100 ppm (bottom) concentration by Exiguobacterium profundum MAK1 over 10 days.

Table 3. Percentage degradation of profenofos by E. profundum MAK1 over 10 days

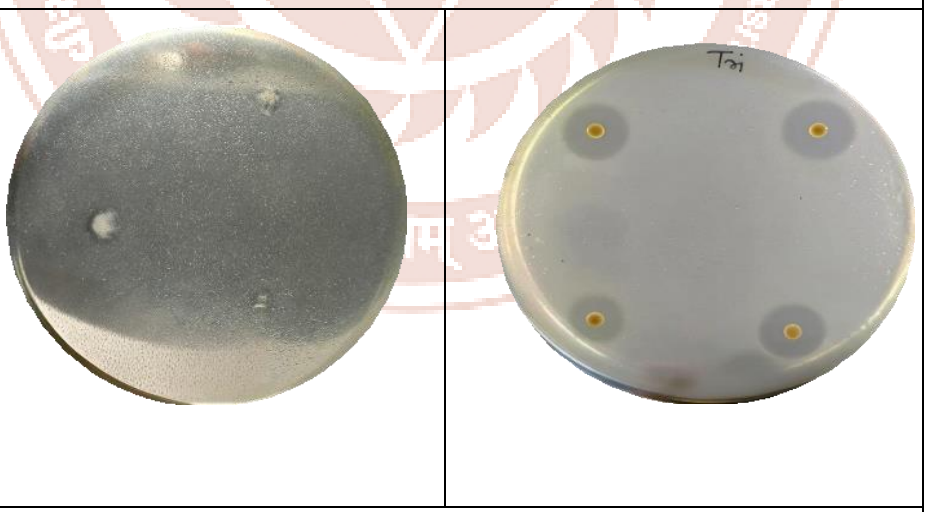
Concentration	Day 1	Day 3	Day 5	Day 7	Day 10
50 ppm	32%	38.5%	45.3%	55.9%	78.4%
100 ppm	1.2%	4.76%	8.1%	15.43%	27.03%

3.5 Esterase Enzyme Activity

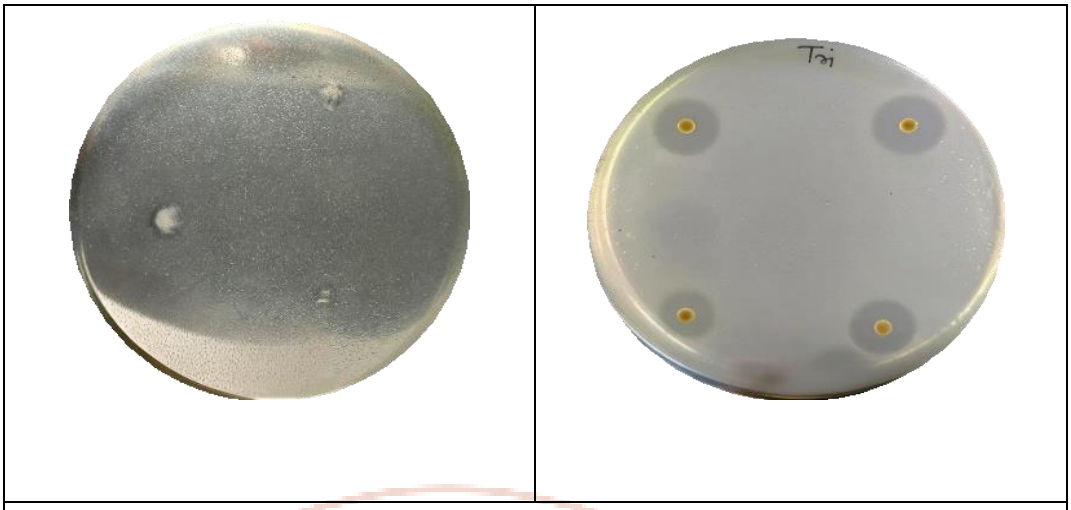
On Tributyrin Agar (TBA) supplemented with 50 ppm profenofos, Exiguobacterium profundum MAK1 produced distinct clear halo zones (zones of clearance) around colonies, confirming active esterase enzyme production by metabolizing lipid in the presence of this specific insecticide (Figure 10). This positive result indicates that the bacterium produces esterase enzymes capable of hydrolyzing tributyrin even under profenofos stress, suggesting high tolerance and possibly active utilization of profenofos as a metabolic substrate. In contrast, esterase activity was reduced in paraquat-supplemented plates, suggesting that paraquat at 50 ppm may inhibit esterase secretion or that the herbicide might disrupts lipid metabolism of the species.



Exiguobacterium sp. cultured on Tributyrin agar containing: Paraquat 50 ppm concentrations



Exiguobacterium sp. cultured on Tributyrin agar containing: Profenofos 50 ppm concentrations



Exiguobacterium sp. cultured on Tributyrin agar containing: Profenofos 50 ppm concentrations

Figure 9 & 10. Esterase activity of Exiguobacterium profundum MAK1 on Tributyrin Agar. (Top) Paraquat 50 ppm – reduced/absent halo zone. (Bottom) Profenofos 50 ppm – clear halo zones indicative of active esterase production.

3.6 Phytotoxicity Assay

The phytotoxicity assay using Vigna radiata revealed significant differences in seed germination and seedling vigor among treatments (Table 4 & 5; Figures 11).). Seeds treated with degraded pesticides showed better germination and seedling growth compared to untreated pesticide samples. Control seeds showed the highest germination (76.6%) and SVI-I (797.41). Seeds watered with treated (biodegraded) paraquat and profenofos yielded 70% germination (SVI-I of 694.4) and 66.6% germination (SVI-I: 535.46) showed while untreated paraquat and profenons caused a notable reduction to 50% germination and SVI-I of 339.5, 46.6% germination in untreated profenofos (SVI-I: 211.10).These results revealed that reduced germination and lower vigor indices (339.5 and 211.09), indicating higher phytotoxicity. It also suggests that pesticide degradation significantly reduced toxicity and improved seed germination and seedling growth in treated paraquat and profenofos.

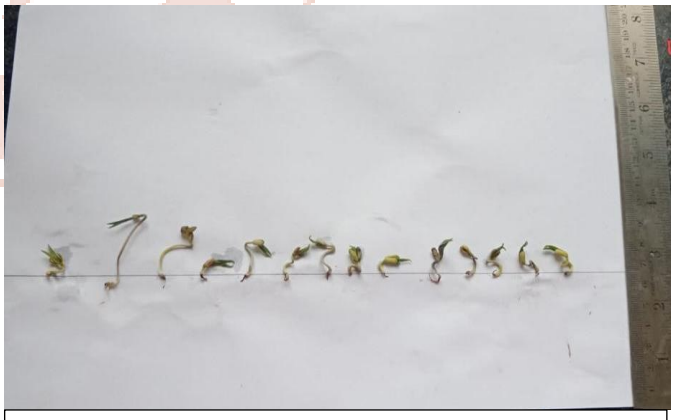
Table 4: . ***Phytotoxicity assay showing germination of Vigna radiata seeds over 0, 3, and 5 days under control, treated profenofos, untreated profenofos, treated paraquat, and untreated paraquat conditions.***

	Control	Treated profenofos	Untreated profenofos	Treated paraquat	paraquat Untreated
0 Day					

3 Days					
5 Days					

Seedling growth of Vigna radiata under control conditions in the phytotoxicity assay

Seedling growth of *Vigna radiata* under Treated paraquat conditions in the phytotoxicity assay



Seedling growth of *Vigna radiata* under Untreated paraquat conditions in the phytotoxicity assay

Seedling growth of *Vigna radiata* under Treated Profenofos conditions in the phytotoxicity assay.

Seedling growth of *Vigna radiata* under Untreated Profenofos conditions in the phytotoxicity assay.

Figure 11. Comparative seedling growth of Vigna radiata: control seedlings (top) showing healthy, elongated root and shoot development, treated paraquat and untreated paraquat (middle) showing healthy elongated and no growth, treated while untreated profenofos (bottom) showing healthy elongated shoots and little growth.

Table 5. Influence of pesticide treatments on germination and seedling vigour of Vigna radiata

Treatment	Germ. %	Shoot (cm)	Root (cm)	Seedling (cm)	SVI-I
Control	76.6	6.61	3.80	10.41	797.41
Treated Paraquat	70.0	8.19	1.73	9.92	694.40
Untreated Paraquat	50.0	2.79	4.00	6.79	339.50
Treated Profenofos	66.6	5.04	3.00	8.04	535.46
Untreated Profenofos	46.6	2.72	1.81	4.53	211.10

3.7 Microbial Inhibition Test

The antimicrobial activity of untreated and biodegraded pesticide samples was assessed against Staphylococcus aureus and Escherichia coli (Table 6; Figures 12 and 13). Controls showed no inhibitory growth (0 mm). Untreated paraquat showed the largest zones of inhibition: 26 mm against Staphylococcus aureus and 30 mm against Escherichia coli.

After microbial degradation (treated paraquat), inhibition zones were reduced to 20 mm (S. aureus) and 21 mm (E. coli), revealing crucial conversion of harmful toxic pesticide compounds to less harmful products.. For profenofos, untreated samples gave inhibition zones of 21 mm (S. aureus) and 28 mm (E. coli), which decreased to 16 mm and 20 mm, respectively, following biodegradation. Escherichia coli showed the highest susceptibility to both pesticides, while Staphylococcus aureus was comparatively less sensitive. .These results suggest that treatment reduces antimicrobial effectiveness and that susceptibility varies among the microorganisms tested.

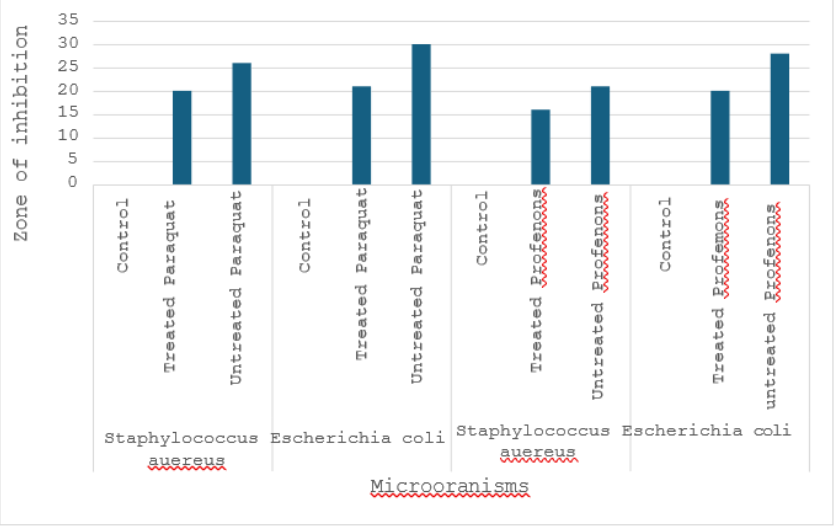
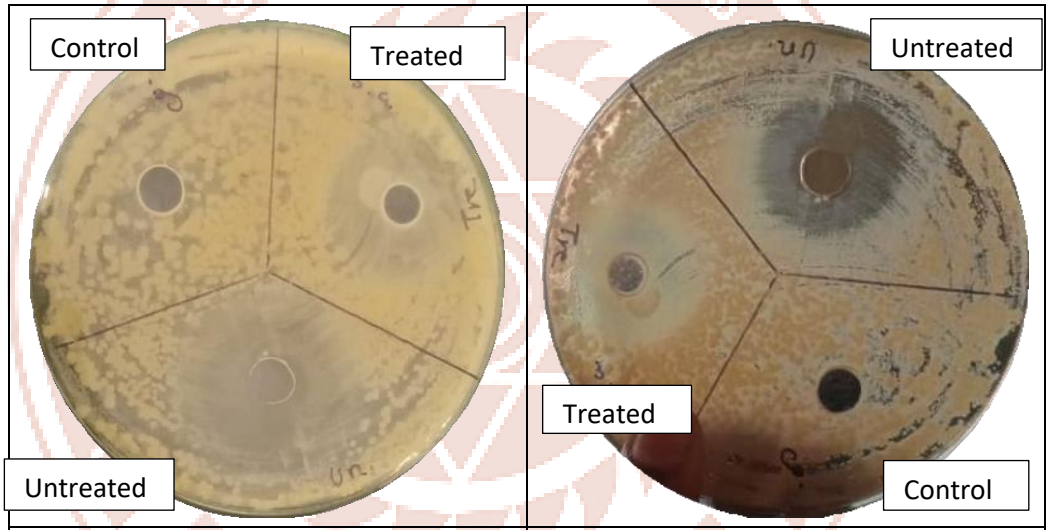
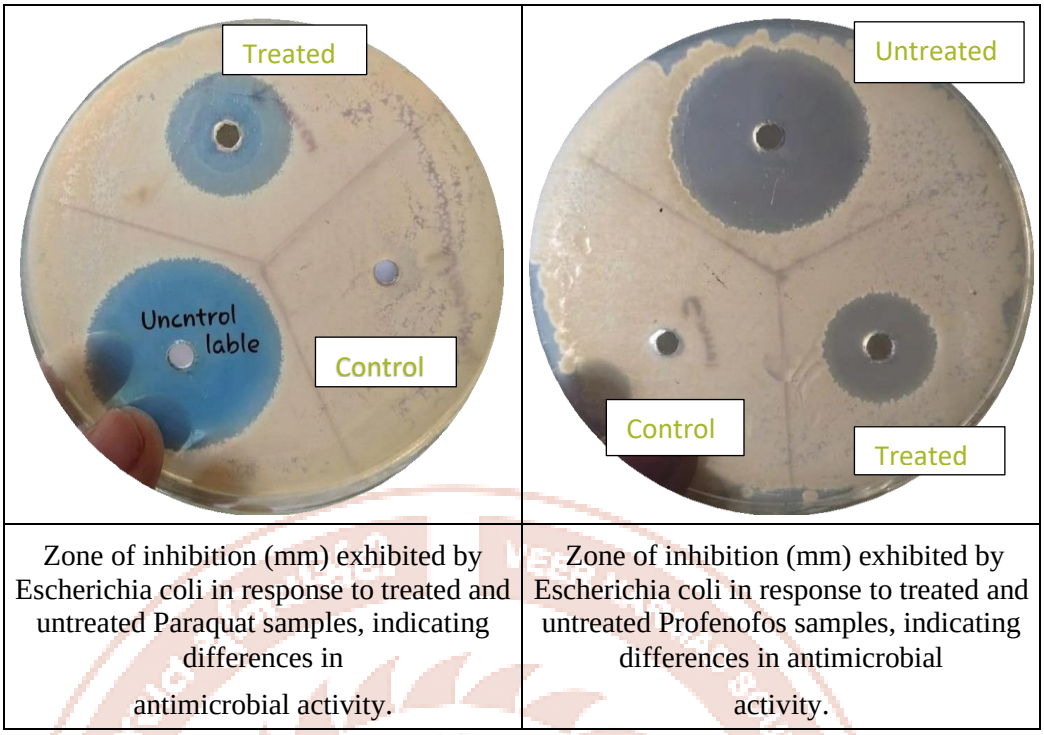


Figure 12. Comparative zone of inhibition (mm) of control, treated, and untreated paraquat and profenofos samples against Staphylococcus aureus and Escherichia coli.



Zone of inhibition (mm) exhibited by *Staphylococcus aureus* in response to treated and untreated Paraquat samples, indicating differences in antimicrobial activity.

Zone of inhibition (mm) exhibited by *Staphylococcus aureus* in response to treated and untreated Profenofos samples, indicating differences in antimicrobial activity.



Zone of inhibition (mm) exhibited by Escherichia coli in response to treated and untreated Paraquat samples, indicating differences in antimicrobial activity.

Zone of inhibition (mm) exhibited by Escherichia coli in response to treated and untreated Profenofos samples, indicating differences in antimicrobial activity.

Figure 12. Agar well diffusion plates showing zones of inhibition of S. aureus & E.coli (left: paraquat treated vs untreated; right: profenofos treated vs untreated).

Table 5. Zone of inhibition (mm) of treated and untreated pesticide samples against S. aureus and E. coli

Microorganism	Control	Treated Paraquat	Untreated Paraquat	Treated Profenofos	Untreated Profenofos
Staphylococcus aureus	0 mm	20 mm	26 mm	16 mm	21 mm
Escherichia coli	0 mm	21 mm	30 mm	20 mm	28 mm

3.8 Plant Growth-Promoting Traits

3.8.1 *Indole-3-Acetic Acid (IAA) Production*

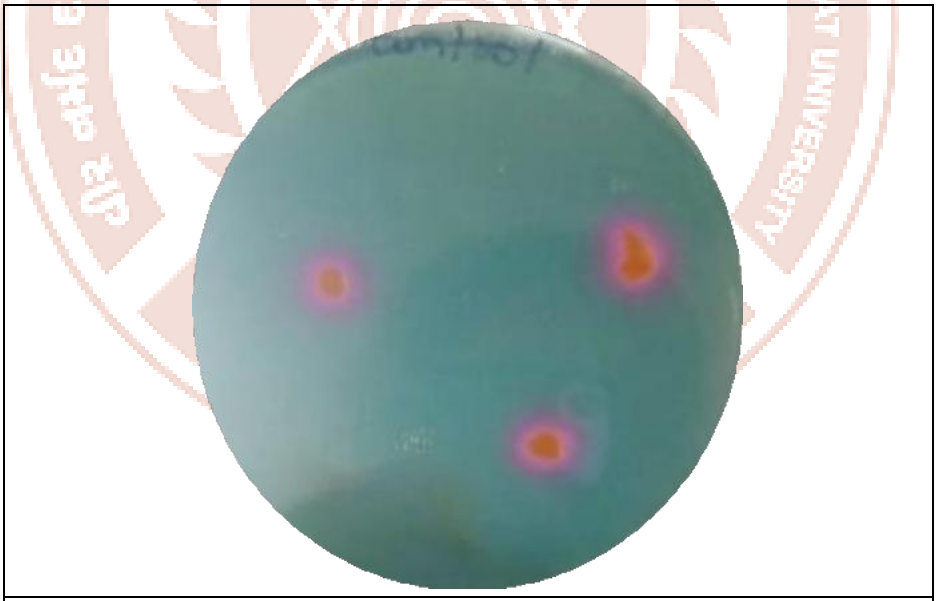
IAA production was measured by optical density at 530 nm using Salkowski's reagent. The control showed an average OD of 0.255. Treated paraquat (OD: 0.230) and profenofos (OD: 0.238) exhibited higher OD values than untreated paraquat (OD: 0.133) and profenofos (OD: 0.145), indicating improved IAA production capability in degraded samples. This suggests that the microbial degradation reduced pesticide toxicity and supported bacterial metabolic activity responsible for pesticide degradation is consistent with preserved or enhanced phytohormone synthesis, and that the degradation products are less inhibitory to IAA biosynthetic pathways.

Table 6. Optical density (OD_{530}) values for IAA production under different pesticide treatments

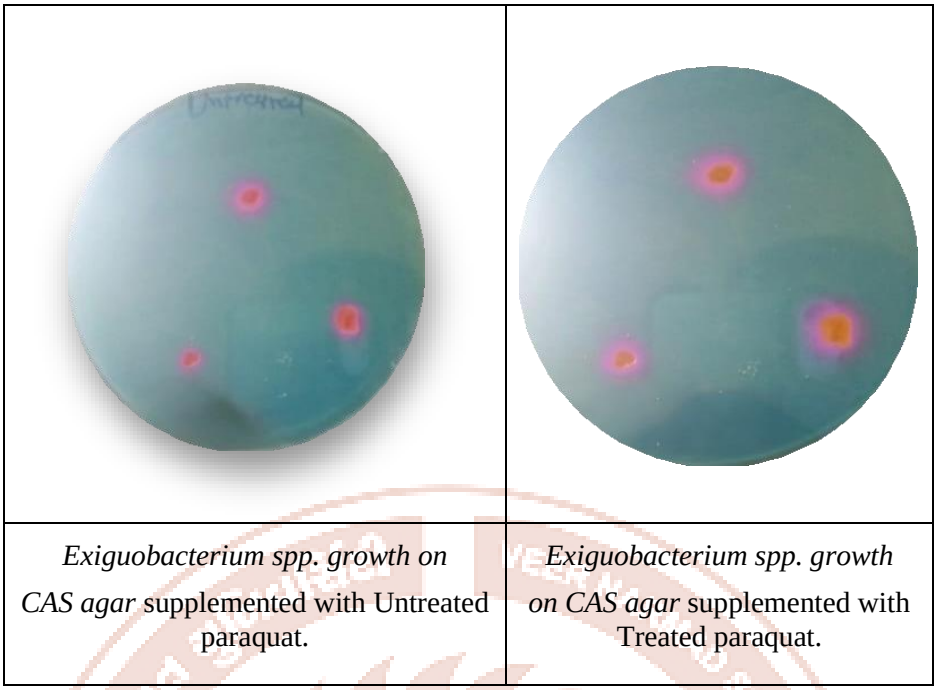
Treatment	OD_1	OD_2	OD_3	Average OD_{530}
Control	0.323	0.231	0.212	0.255
Treated Paraquat	0.295	0.205	0.189	0.230
Untreated Paraquat	0.148	0.132	0.119	0.133
Treated Profenofos	0.301	0.214	0.198	0.238
Untreated Profenofos	0.165	0.142	0.128	0.145

3.8.2 *Siderophore Production*

Exiguobacterium profundum MAK1 produced distinct orange/pink halo zones on Chrome Azurol S (CAS) agar, confirming active siderophore synthesis (Figure 13). Siderophore production was maintained in both paraquat- and profenofos-supplemented CAS plates, demonstrating that the bacterium retains iron-chelating activity even under pesticide stress. Variations in halo intensity between treated and untreated pesticide supplemented plates suggest the variation in siderophore production under different stress conditions. These supplements may influence the level of siderophore production compared to the control.



Control plate of *Exiguobacterium* spp. on CAS agar without pesticide supplementation



Exiguobacterium spp. growth on CAS agar supplemented with Untreated paraquat.

Exiguobacterium spp. growth on CAS agar supplemented with Treated paraquat.

Figure 13. Siderophore production by Exiguobacterium profundum MAK1 on Chrome Azurol S (CAS) agar. (Top) Control plate without pesticide. (Bottom left) CAS agar with untreated paraquat. (Bottom right) CAS agar with treated paraquat. Orange/pink halos indicate active siderophore synthesis.

3.8.3 *Ammonia Production*

Ammonia production was assessed by optical density measurement following Nessler's reagent treatment. Control samples showed an average OD of 3.00. Treated samples exhibited slightly lower OD values compared to untreated ones (treated paraquat: 2.91 vs untreated: 3.03; treated profenofos: 2.84 vs untreated: 3.01). It suggests the possible degradation or transformation of the pesticides by microbial activity consistent with ammonia production capacity being retained and pesticide degradation altering the nitrogen metabolism system within the Exiguobacterium Species.

Table 7: Comparison of optical density (OD) values for ammonia production by Exiguobacterium sp. in control, treated, and untreated paraquat and profenofos samples.

Sr no.	Pesticide name	OD1	OD2	OD3	Average
1	Control	3.14	2.858	3.004	3.000667
2	Treated paraquat	2.95	2.88	2.91	2.913333

3	Untreated paraquat	3	2.97	3.12	3.03
4	Treated profenofos	2.86	2.82	2.84	2.84
5	Untreated profenofos	3.02	2.99	3.01	3.006667

4. Discussion

The current study showed that the Exiguobacterium profundum isolate MAK1 can break down both paraquat and profenofos while still promoting plant growth and maintaining enzymatic activities. Molecular identification using 16S rRNA sequencing revealed a 100% similarity with Exiguobacterium profundum strain 0056 (KP236196.1). This species was first described by Crapart et al. (2007) as a moderately thermophilic and salt-tolerant bacterium found in deep-sea hydrothermal vents. The isolation of E. profundum from polluted soil near Ankleshwar, Gujarat, highlights the adaptability of the genus Exiguobacterium, which is known for its environmental resilience and importance in biotechnology (Crapart et al., 2007; Kasana & Pandey, 2018).

MAK1 showed several traits that promote plant growth, including the production of indole-3-acetic acid (IAA), ammonia and siderophores. Similar traits have been found in other pesticide-degrading Exiguobacterium species, where they helped to improve plant growth and stress tolerance in contaminated conditions (Sur & Sathiavelu, 2024). Species in the Exiguobacterium genus are recognized for their ability to both remediate environments and promote plant growth (Kasana & Pandey, 2018).

The isolate demonstrated better growth and degradation efficiency at 50 ppm compared to 100 ppm of both pesticides. This suggests concentration-dependent substrate degradation efficiency , which is common in pesticide-degrading microorganisms (Subsanguan et al., 2020). Paraquat degradation by MAK1 reached 92.32% at 50 ppm and 80.37% at 100 ppm in 10 days, which is comparable to degradation efficiencies reported in other bacterial systems under optimized conditions (Huang et al., 2019; Jindakaraked et al., 2023). Profenofos degradation was 78.4% at 50 ppm within 10 days, which aligns with results found in other profenofos-degrading bacteria Mahajan et al. (2021) reported that Bacillus spp. PF1 degraded approximately 93% profenofos over 30 days under optimized incubation conditions, whereas

Fang et al. (2022) observed degradation of profenofos in soil systems by Cupriavidus nantongensis X1T. Although direct comparison between flask and soil systems is limited by differing environmental conditions, the degradation efficiency observed in the present study indicates that MAK1 possesses considerable biodegradation potential .The lower degradation at higher pesticide concentrations may result from toxic effects on microbial metabolism and degrading enzymes.

During profenofos degradation, esterase activity was observed, indicating the role of hydrolytic enzymes in the biodegradation process . Sur & Sathiavelu (2024) similarly noted versatile enzymatic activity in rhizospheric Exiguobacterium for dimethoate degradation, supporting the role of esterases as key bioremediation enzymes in this genus.Previous studies have reported similar esterase-mediated degradation pathways involving organophosphorus hydrolases like OpdB (Fang et al., 2022). The reduced esterase activity under paraquat stress may relate to oxidative stress caused by reactive oxygen species generated by paraquat exposure . As paraquat-induced ROS has been shown to directly suppress esterase-class enzyme activity (Ramalho et al., 2025) through oxidation of critical protein residues (Jomova et al., 2024).

Phytotoxicity analysis using Vigna radiata indicated a significant increase in seed vigor after microbial treatment compared to untreated pesticide controls, showing reduced toxicity following degradation. These results are consistent with reduced phytotoxicity of degradation products compared to parent pesticide molecules, as also observed by Sillapawattana & Klungsupya (2022) for fungal paraquat metabolites. The decline in antimicrobial activity against Staphylococcus aureus and Escherichia coli further supported the detoxification of pesticide-containing samples. Aftab et. al, (2011); reported that biodegraded product was non-toxic to beneficial soil bacteria.

The overall findings suggest that E. profundum MAK1 has promising potential for simultaneous bioremediation of pesticides and promotion of plant growth in contaminated agricultural settings. However, further studies involving GC–MS/HPLC analysis and genomic characterization are necessary to identify the degradation intermediates and clarify the molecular mechanisms involved.

5. Conclusion

The present study establishes Exiguobacterium profundum (isolate MAK1) as an effective pesticide-degrading bacterium capable of significantly reducing the concentrations of both paraquat (up to 92.32% at 50 ppm) and profenofos (up to 78.4% at 50 ppm) over a 10-day incubation period under laboratory conditions. Molecular identification by 16S rRNA

sequencing confirmed 100% identity with E. profundum strain 0056. Esterase enzyme activity confirmed enzymatic involvement in pesticide breakdown, particularly for profenofos. Phytotoxicity assays and microbial inhibition tests validated that microbial treatment substantially reduced the toxicity of both pesticides. Additionally, Exiguobacterium profundum MAK1 demonstrated valuable PGP traits—IAA production, siderophore synthesis, and ammonia production—establishing it as a dual-function bioremediation and plant growth-promoting agent. These findings support that pesticide biodegradation is both time- and concentration-dependent, and establish the application of E. profundum in bioaugmentation strategies for pesticide-contaminated agricultural soils and provide an evidence for future field-applied bioremediation studies, particularly for paraquat.

6. Future Prospects

Future research should focus on large-scale and field-level biodegradation studies to evaluate the efficiency and stability of Exiguobacterium profundum MAK1 under natural soil and environmental conditions; whole genome sequencing, gene expression analysis, alongwith proteomic studies to identify the specific catabolic genes, metabolic pathways, and esterase variants responsible for paraquat and profenofos degradation, advanced analytical characterisation of degradation bioproducts must be analyzed by GC–MS and HPLC to confirm complete detoxification and elucidate metabolic pathways; optimisation of environmental parameters like pH, temperature, nutrient availability, and inoculum density to maximise it's in situ bioremediation efficiency; development of immobilised cell or carrier-based formulations to improve bacterial survival and degradation stability in complex soil environments; ecotoxicological assessments including soil microbial diversity analysis and long-term plant growth experiments to confirm environmental safety and also development of biofertilizer or bioremediation formulations containing MAK1 for simultaneous enhancement of soil fertility, promotion of plant growth, and remediation of pesticide contamination in agricultural environments.

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