Iraqi Journal of Market Research and Consumer Protection
(2026) 18(2): 310-323.
DOI:
Hameed & Hassan

المجلة العراقية لبحوث السوق
وحماية المستهلك

ISOLATION AND IDENTIFICATION OF INDIGENOUS BACTERIA USING PCR AND EVALUATION OF THEIR EFFICIENCY IN DEGRADING PYROXSULAM PESTICIDE IN LIQUID CULTURE MEDIA

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Received 1/ 7/ 2025, Accepted 1/ 9/ 2025, Published 30/ 6/ 2026

ABSTRACT

This study aimed to isolate and identify indigenous bacterial strains from soils contaminated with the herbicide pyroxsulam and to assess their ability to degrade the compound in liquid culture media. Soil samples were collected from wheat fields previously treated with the herbicide. Several bacterial isolates were obtained and subjected to morphological characterization and biochemical tests. Identification was further confirmed using polymerase chain reaction (PCR) analysis. The most effective isolate was identified as *Paenibacillus alvei* based on the results. The degradation capacity of the isolate was determined at three concentrations (36, 52.6 and 112 mg L^{-1}) of the herbicide in nutrient broth. Growth of bacteria was observed for different incubations. The isolate grew in all herbicide concentrations. These demonstrates unequivocal the potential of the isolate to degrade pyroxsulam and thus its applicable potential in the bioremediation of polluted soil.

Keywords: *Paenibacillus*, bioremediation, Diagnosis.

عزل وتشخيص بكتريا محلية باستعمال تقنية PCR واختبار كفاءتها في تحلل مبيد Pyroxsulam في الأوساط الزرعية السائلة

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الخلاصة

اجري هذا البحث بهدف عزل وتشخيص بكتريا محلية من ترب ملوثة بمبيد pyroxsulam، ودراسة قدرتها على تحليل المبيد في الأوساط السائلة. جمعت عينات التربة من حقول مزروعة بالحنطة ومعاملة بالمبيد. تم الحصول على عدة عزلات بكتيرية، خضعت للفحص المظهري والاختبارات البيوكيميائية، ثم تم تأكيد التشخيص باستعمال تقنية تفاعل البلمرة المتسلسل (PCR)، وأثبتت النتائج ان العزلة الأكثر كفاءة تعود الى جنس *Paenibacillus alvei*. اختبرت كفاءة العزلة في تحليل المبيد باستعمال ثلاث تراكيز مختلفة (36 و52.6 و112) ملغم لتر$^{-1}$ في أوساط غذائية سائلة، وتم تتبع منحنى النمو البكتيري خلال فترات حضن مختلفة، أظهرت النتائج قدرة العزلة على النمو في جميع تراكيز المبيد.

[*]**This article is taken from the doctoral dissertation of the first researcher.**

تشير هذه النتائج الى امتلاك العزلة قدرة واضحة على تحليل المبيد مما يؤكد إمكانية استعمالها في تقنيات المعالجة الحيوية للترب الملوثة بـ pyroxsulam.

الكلمات المفتاحية: Paenibacillus ، المعالجة الحيوية، التشخيص.

INTRODUCTION

Pesticide pollution is one of the most significant environmental problems while affecting the ecosystems as well as biological systems (**Ullah *et al.*, 2024**). Pesticides play a crucial role in modern agriculture by protecting crops from pests, diseases, and weeds, thereby ensuring food security and increasing agricultural productivity (**Gupta,2023**) Pesticides are indispensable for protecting crops, but the over-use and misuse of pesticides represents the most serious threat for the health of ecosystems and the conservation of biodiversity. More specifically, they can negatively impact beneficial Indigenous soil microorganisms, the composition and activity of which can become affected **(Singh *et al.*, 2016)**. Pesticides affect microbial communities either directly or indirectly. Direct effects include microbial death through the inhibition of metabolic processes, while indirect effects arise from their impact on soil or host organisms. Some pesticides interfere with plant cellular metabolism, such as photosynthesis and phytohormone biosynthesis, which in turn alters plant traits and affects associated microbial communities **(Fuchs *et al.*, 2021)**. Among these pesticides, Pyroxsulam is a selective systemic herbicide widely used for weed control in wheat fields. It belongs to the triazolopyrimidine sulfonamide group, which works by inhibiting the acetolactate synthase (ALS) enzyme, which is responsible for synthesizing amino acids necessary for cell division **(Jursik *et al.*, 2016)**. Pyroxsulam degrades slowly in soil and is relatively mobile, with a half-life ranging between 3 and 23 days. This increases its potential to accumulate in soil and leach into water and air, thereby affecting non-target organisms, especially beneficial soil microorganisms **(Kim *et al.*, 2017; Bi *et al.*, 2020)**. High concentrations of the herbicide inhibit nitrification by inhibiting the activity of organisms responsible for nitrification. It also negatively impacts nitrogen fixation, which negatively impacts soil fertility and, consequently, agricultural production (**Grey *et al.*, 2017**).

With such concerns, bioremediation has become a viable and sustainable strategy to cleanup or eliminate toxic chemical pollutants from the environment. This treatment system involves the use of a range of microorganisms including bacteria, fungi, algae and yeasts in pollutant degradation **(Li *et al.*, 2018).** The genera of microorganisms most commonly used in bioremediation are Bacillus, Nitrosamines, Pseudomonas, Mycobacterium and Achromobacter etc **(Enerijiofi, 2021)**. Bioremediation is accomplished through degradation of pollutants by microorganisms, using them as substrates for energy or nutrients. This is done by breaking down complex substances chemically. This ultimately alters the primary compounds and materials into less toxic or toxic-free secondary compounds and finally into stable non-toxic end products, such as carbon dioxide and water. This is the basis of bioremediation **(Sharma & Kumar, 2021).** In a study conducted by **Hatem *et al.* (2015)**, it was shown that various bacterial species can be isolated and identified from agricultural soils. This finding represents an initial step toward understanding the role of microbial organisms in the remediation of various environmental pollutants, including pesticide residues. Microorganisms employ various mechanisms, including metabolism, assimilation, fermentation, and enzymatic degradation,

with the latter being a primary mechanism used by microorganisms in bioremediation **(Das &Dash, 2014).** Several studies, including the study by **Ali *et al*. (2016)**, have indicated that Iraqi soils are exposed to negative impacts resulting from the use of agricultural pesticides and industrial waste. This necessitates interventions such as the use of bioremediation to reduce the harmful environmental effects in combating pesticide pollution.

This research aims to isolate and identify local bacteria from soils treated with pyroxsulam herbicide, test the efficiency of the isolates in analyzing the pesticide in liquid media at different concentrations of the herbicide, study the growth curves of the most efficient bacterial isolate under the influence of the herbicide, and determine the minimum growth inhibitory concentration (MIC) and the lethal concentration (MBC) for the selected isolate.

MATERIALS AND METHOD

This laboratory study was performed in Agricultural Research Department / Ministry of Agriculture lab for the academic year 2023-2024. It was undertaken with the aim to isolate and characterize bacterial isolates from the pyroxsulam treated soils followed by their analysis for biological efficiency to degrade the herbicide in liquid medium. Statistical analyses were conducted by ANOVA and averaged compared with the LSD test at 0.05 level of probability **(Marini, 2003; Piepho *et al*., 2006)**.

1. Soil Sampling and Bacterial Isolation

A total of eight soil samples were collected from a depth of 0–30 cm in the rhizosphere zone of wheat fields previously treated with pyroxsulam in various locations within the Nahrawan region. These samples were collected to isolate potential herbicide-tolerant or -adapted bacterial strains capable of degrading pyroxsulam. The samples were transported to the laboratory under sterile conditions. Serial decimal dilutions were prepared using physiological saline solution (0.85% NaCl), and the dilutions were plated on nutrient agar medium (Nutrient Agar) in triplicate. The plates were incubated at 30 °C for 72 hours, resulting in the emergence of various bacterial colonies. Sub-culturing on fresh nutrient agar was performed to obtain pure colonies for bacterial identification.

Morphological examination and Gram staining were conducted on the colonies. Colonies were selected based on distinct morphological characteristics, including shape, color, and size. Based on these criteria, eight morphologically distinct bacterial isolates were successfully isolated and purified **(Uqab *et al*., 2016; Singh & Walker, 2006).**

2. Evaluation of Bacterial Isolates' Efficiency in Pesticide Degradation

Each bacterial isolate was incubated in Nutrient Broth medium containing pyroxsulam at three concentrations (36, 56.2, and 112 mg L^{-1}) for 14 days at 30°C. After the incubation period, the supernatant was separated using a centrifuge, and the residual pesticide concentration was measured using High-Performance Liquid Chromatography (HPLC) following the QuEChERS method **(Anastassiades *et al*., 2003).** Isolate C4 was selected for further study due to its superior efficiency in degrading the pesticide compared to other isolates.

3. Identification of C4 Isolation

The C4 bacterial isolate was cultured. The isolate was cultured on Nutrient Agar and incubated at 30°C for 24–48 hours. Colonies were characterized in terms of color, shape, texture, margins, and colony surface. Microscopic examination of the bacterial isolates growing on nutrient agar was performed after a bacterial smear was placed on a clean, sterile glass slide and stained with several Gram stains. The response to the Gram stain was recorded as either Gram-negative or Gram-positive **(Daane *et al*., 2002)**. Subsequent biochemical tests were carried out namely: catalase, oxidase, gelatinolysis, indole test, lactose fermentation, motility test, and starch hydrolysis and citrate uptake by test tube and suitable nutrient media by a standard procedure **(Tortora *et al*., 2018; Ye *et al*., 2012)**. Molecular identification of the bacterial isolate. The bacterial isolate was identified using molecular techniques, the 16S rRNA gene was amplified by polymerase chain reaction (PCR) with specific primers. The reaction results were sent to Macrogen Inc. (South Korea/Seoul) for forward and reverse sequencing according to the company's instructions. The base sequences were then analyzed using BioEdit v7.1 software and compared to the NCBI database using the BLASTn engine to determine the molecular identity of the isolate **(Zhang *et al*., 2000; Pelczar *et al*., 1993).**

4. Bacterial Growth Curve

The bacterial growth curve was measured using the standard plate count method. Test tubes containing 125 ml of liquid nutrient broth were inoculated with the selected bacterial isolate. Then, the pesticide pyroxsulam was added at three concentrations (36, 56.2, and 112) mg/L in three replicates. The cultures were incubated at 30°C. One ml of each test tube was transferred to Petri dishes, which were added by surface diffusion. The bacterial count (CFU/ml) was calculated every 24 hours for 10 days. The results were used to plot bacterial growth curves under the influence of different pesticide concentrations and compared to the control treatment **(Balouiri *et al*., 2016).**

5. Determination of Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC)

The Well Diffusion Method was employed to determine the MIC and MBC of pyroxsulam against the selected bacterial isolate **(Atlas & Bartha, 1998)**. Muller-Hinton agar plates were inoculated with 1 mL of bacterial suspension, and 6 mm diameter wells were created using a sterile cork borer. Each well received 100 µL of pyroxsulam at concentrations ranging from 36 to 500 mg L^{-1}. Plates were incubated at 37°C for 24 hours.

The MIC was determined based on the lowest concentration around which a clear inhibition zone appeared, i.e., the lowest concentration at which no growth of bacterial colonies appeared. The lethal concentration (MBC) was determined by taking samples from the holes in which no growth appeared and re-seeding them on a pesticide-free medium, then incubating again. The concentration at which no growth appeared was considered later growth is MBC.

RESULTS AND DISCUSSION

1. Morphological characteristics of bacterial isolates from soil samples treated with pyroxsulam.

The 8 bacterial isolates ButC1–ButC8, are detailed in Table (1), showed impressive diversity within strains (round, rhizoid and irregular), colors, margin type and consistency. This

discrepancy reflects the intricate soil system of the samples. Moreover, the result of Gram staining indicated variability in the Gram reaction of the isolates (some were Gram-positive and some were Gram-negative) reflecting dissimilarities in cell wall composition. The differences in morphological characteristics and Gram staining represented an important step in the initial classification of these bacterial colonies isolated from agricultural fields and contributed to supporting their selection for subsequent evaluation of their efficiency in degrading pyroxsulam.

Table (1): Morphological characteristics of bacterial colony types isolated from soil samples treated with Pyroxsulam.

Bacterial Isolate	Gram Stain	Colony Color	Colony Shape	Colony Texture	Colony Margin	Colony Size
C1	+	Creamy white	Circular	Dry	Wavy	Medium
C2	+	White	Circular	Rough dry	Wavy	Large
C3	-	Grayish white	Circular	Smooth sticky	Regular	Medium
C4	+	Creamy white	Circular	Rough dry	Irregular	Large
C5	+	White	Circular	Smooth sticky	Slightly wavy	Very large
C6	-	Yellowish white	Circular	Mucous smooth	Regular	Medium
C4	+	Creamy	Rod-shaped	Mucous	Irregular	Small
C8	-	White	Circular	Sticky	Regular	Medium

Efficiency of Bacterial Isolates in Degrading Pyroxsulam in Liquid Media

The results presented in Table (2) show variations among the bacterial isolates (C1–C8) in their ability to degrade pyroxsulam in liquid culture media. The residual concentrations of the herbicide were determined using High-Performance Liquid Chromatography (HPLC), by comparing the retention time of the compound in the tested samples with that of the standard reference. The results indicated that the locally isolated bacterial strain C4 significantly outperformed all other isolates in terms of its degradation efficiency across all three tested concentrations of pyroxsulam: 36, 52.6, and 112 mg L^{-1}. It recorded the lowest average residual concentration of 19.1 mg L^{-1} compared to other isolates. At the first concentration level (H1), the initial concentration of 36 mg L^{-1} was reduced to 2.2 mg L^{-1}, representing a degradation rate of 93.89% after 14 days of incubation. The second concentration (H2) decreased from 52.6 to 14.5 mg L^{-1}, corresponding to a degradation efficiency of 72.44%. For the highest concentration (H3), the level dropped from 112 to 40.5 mg L^{-1}, achieving a degradation rate of 63.84%. The degradation was quantified using HPLC following the QuEChERS extraction method **(Anastassiades *et al*., 2003)**

Table 2. Degradation of Pyroxsulam by different bacterial isolates in liquid media (mg L^{-1}).

Isolate	Pyroxsulam (mg L^{-1}).			Mean isolate
	36	52.6	112(H3)	
Control	34.6	50.0	107.5	64.0
C1	5.2	21.0	58.0	28.1
C2	4.3	19.6	56.1	26.7
C3	4.1	17.8	54.8	25.6
C4	2.2	14.5	40.5	19.1
C5	4.3	20.4	52.1	25.6
C6	4.4	19.7	50.8	24.9
C7	6.0	25.0	58.1	29.7
C8	5.3	22.2	55.5	27.7
Mean Herbicide	7.8	23.3	59.3	
LSD 0.05	Isolate	Herbicide	Interaction	
	0.63	0.36	1.08	

The results, as shown in Figure (1, 2, 3, and 4), showed that the retention time of the standard herbicide pyroxsulam was 5.08 minutes and the retention time of the sample (C4 bacteria + 36 mg L^{-1} of herbicide) was 5.05 minutes, which is very close to the retention time of the standard herbicide, while the retention time of the sample for the two concentrations (52.6 and 112) mg L^{-1} of herbicide was 5.08 and 5.03 minutes, respectively. Therefore, the C4 isolate was selected for its efficiency in degrading the herbicide compared to the other isolates, and then it was subjected to diagnosis to determine the type of this bacteria by studying the morphological and microscopic characteristics of the C4 bacterial isolate.

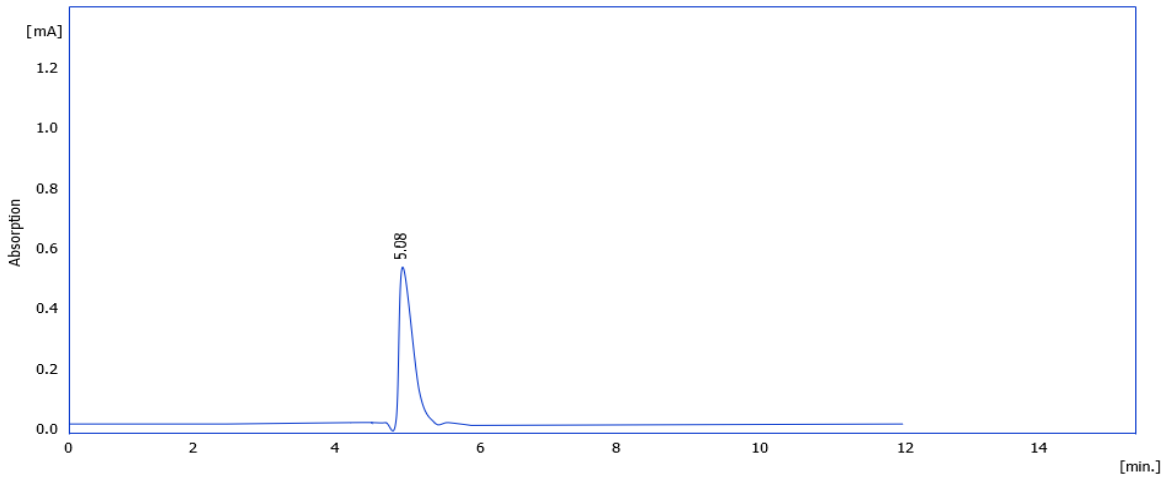

Figure (1): HPLC chromatogram of the Pyroxsulam standard.

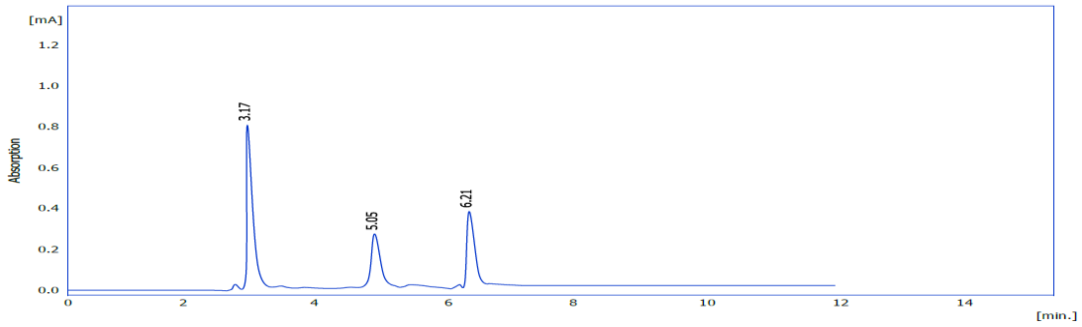

Figure (2): HPLC chromatogram of isolate C4 at concentration H1.

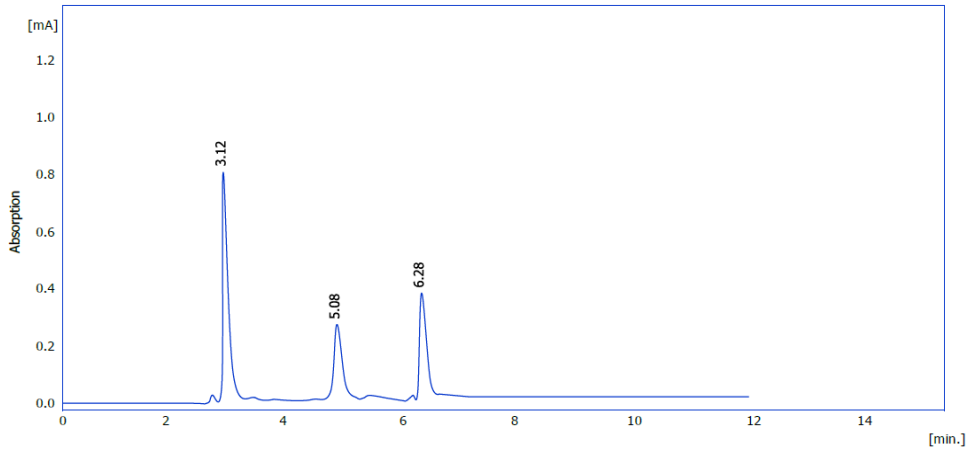

Figure (3): HPLC chromatogram of isolate C4 at concentration H2.

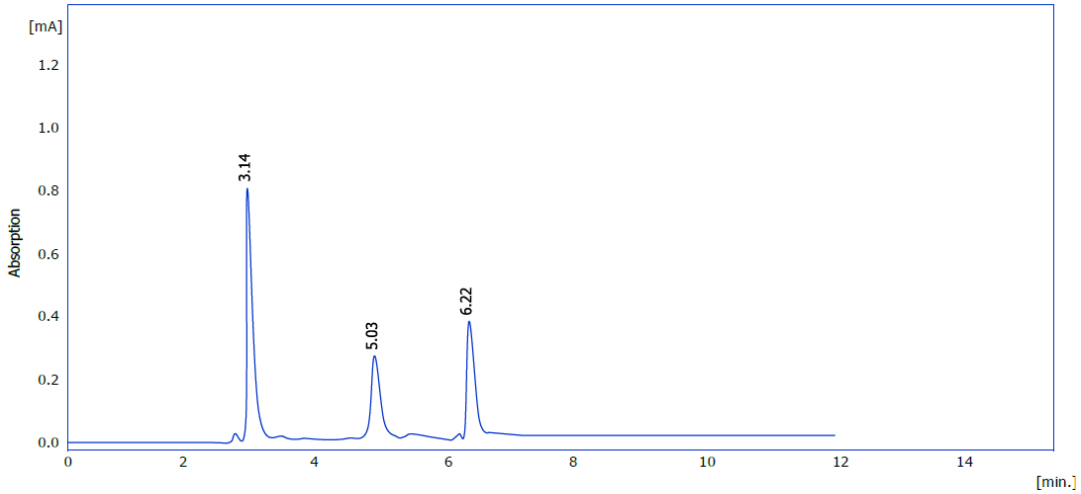

Figure (4): HPLC chromatogram of isolate C4 at concentration H3.

Identification of Bacterial Isolate C4

1. Morphological and Microscopic Characteristics

The results presented in Table (3), which describe the morphological and microscopic features of bacterial colonies grown on nutrient agar, showed that the colonies were large and circular, with diameters ranging from 4 to 7 mm. They exhibited irregular or wavy margins, a creamy white color, dry and non-mucoid consistency, and a rough surface with noticeable protrusions (**Atlas & Bartha, 1998**). Microscopic examination revealed that the bacterial cells were rod-shaped and Gram-positive, occurring singly or in pairs **(Pelczar *et al*., 1993)**.

Table (3): Selected morphological and microscopic characteristics of C4 bacterium.

Test	Bacterium
Gram stain	+
Colony shape	Round
Colony diameter	4–7 mm
Colony color	Creamy white
Colony elevation	Slightly convex
Colony texture	Dry
Colony margin	Irregular and wavy
Cell shape	Rod-shaped
Cell arrangement	Single

II. Biochemical Tests for Isolate C4

The results of the biochemical tests for bacterial isolate C4, detailed in Table (4), showed that the bacterial isolate was positive for citrate utilization, indicated by a color change from green to blue due to the breakdown of citrate and its use as a carbon source. The isolate also showed a positive result for the indole test, demonstrating its ability to break down the amino acid tryptophan via the enzyme tryptophanase, forming a red ring on the surface. A positive catalase test result was observed, indicating its ability to break down hydrogen peroxide into oxygen and water, which was evident as gas bubbles. The isolate also showed a positive result for the oxidase test, due to its ability to change color to dark red. The bacterial isolate also showed a positive result for starch decomposition, as iodine reacted with the starch, forming a dark brown color. The gelatin degradation test results also demonstrated the bacterial isolate's ability to liquefy gelatin and produce gelatinase, as the gelatin medium remained in a liquid state at 4°C. The lactose fermentation result was positive, as the color of the alpha-phenol red dye changed to yellow or yellow-orange as a result of the sugar fermentation and the conversion of the basic medium to acidic. The bacterial isolate also showed a positive result for the motility test **(Cappuccino & Sherman, 2014; MacFaddin, 2000).**

Table (4): Biochemical tests of the bacterial isolate C4.

Test	Result
Citrate utilization	+
Indole	+
Catalase	+
Oxidase	+
Starch hydrolysis	+
Gelatin hydrolysis	+
Lactose fermentation	+
Motility	+

Molecular Identification (PCR)

The results of the 16S rRNA gene sequencing analysis for bacterial isolate C4 showed a high degree of similarity with reference sequences stored in the NCBI database. The BLASTn analysis revealed that the isolate matched *Paenibacillus alvei* (Accession No. MH475939.1) with 99.73% identity, as shown in Figure (5). This clearly indicates that the bacterial isolate belongs to this species **(Zhang *et al.*, 2000; Ye *et al.*, 2012).** The length of the amplified gene segment was approximately 1502 base pairs. Alignment results uncovered the presence of four single nucleotide polymorphisms (SNPs) when comparing the isolate's sequence to the reference sequence. These mutations were confirmed by manual examination of the sequence chromatogram, supporting the reliability of the results obtained from the molecular identification process.

Description	Scientific Name	Max Score	Total Score	Query Cover	E value	Per. Ident	Acc. Len	Accession
Paenibacillus alvei strain RG05 16S ribosomal RNA gene, partial sequence	Paenibacillus alvei	2691	2691	100%	0.0	99.73%	1502	MH475939.1
Paenibacillus alvei strain RG05 16S ribosomal RNA gene, partial sequence	Paenibacillus alvei	2691	2691	100%	0.0	99.73%	1502	KX962163.1
Uncultured bacterium clone 5 16S ribosomal RNA gene, partial sequence	uncultured bacterium	2631	2631	100%	0.0	99.00%	1523	JF830167.1
Paenibacillus alvei DSM 29 16S ribosomal RNA, partial sequence	Paenibacillus alvei DSM 29	2626	2626	99%	0.0	99.00%	1527	NR_042091.1
Paenibacillus alvei strain BPW1 16S ribosomal RNA gene, partial sequence	Paenibacillus alvei	2626	2626	99%	0.0	99.00%	1527	MT482631.1
Paenibacillus sp. RKJ14 16S ribosomal RNA gene, partial sequence	Paenibacillus sp. RKJ14	2620	2620	99%	0.0	99.12%	1499	GQ927307.2
Paenibacillus alvei gene for 16S rRNA, partial sequence	Paenibacillus alvei DSM 29	2613	2613	99%	0.0	98.99%	1501	AB073200.1
Paenibacillus sp. strain BAB-6059 16S ribosomal RNA gene, partial sequence	Paenibacillus sp.	2609	2609	98%	0.0	99.12%	1479	KY672900.1
Paenibacillus alvei isolate Paenibacillus B-LR1 genome assembly, chromosome: PBLR	Paenibacillus alvei	2608	25999	99%	0.0	98.73%	6694765	LS992241.1
Paenibacillus alvei strain V1 16S ribosomal RNA gene, partial sequence	Paenibacillus alvei	2606	2606	99%	0.0	98.73%	1497	EU435385.1
Paenibacillus sp. L5 16S ribosomal RNA gene, partial sequence	Paenibacillus sp. L5	2603	2603	99%	0.0	98.66%	1513	EF426449.1
Paenibacillus alvei strain NBRC 3343 16S ribosomal RNA, partial sequence	Paenibacillus alvei	2603	2603	98%	0.0	99.05%	1476	NR_113577.1
Paenibacillus sp. GT08-03 partial 16S rRNA gene	Paenibacillus sp. GT08-03	2602	2602	99%	0.0	98.59%	1498	AM162320.1
Paenibacillus alvei strain Oa17A 16S ribosomal RNA gene, partial sequence	Paenibacillus alvei	2594	2594	98%	0.0	99.05%	1473	MG461561.1
Paenibacillus alvei partial 16S rRNA gene, isolate CCM2B	Paenibacillus alvei	2590	2590	99%	0.0	98.53%	1522	FN433032.1
Paenibacillus sp. JCM 28274 gene for 16S ribosomal RNA, partial sequence	Paenibacillus sp. JCM 28274	2585	2585	99%	0.0	98.39%	1513	LC133621.2
Paenibacillus sp. JCM 28311 gene for 16S ribosomal RNA, partial sequence	Paenibacillus sp. JCM 28311	2585	2585	99%	0.0	98.39%	1513	LC133658.1
Paenibacillus alvei gene for 16S rRNA, partial sequence, strain: AUG6	Paenibacillus alvei	2574	2574	99%	0.0	98.52%	1484	AB377108.1

Figure (5): Similarities based on the NCBI BLASTn tool between the studied ribosomal sequences of *Paenibacillus alvei* and the ribosomal sequences documented in the NCBI database.

Growth Curve of *Paenibacillus alvei* Exposed to Different Herbicide Concentrations

The bacterial growth curve of *Paenibacillus alvei* exposed to three different concentrations of Pyroxsulam herbicide (36, 52.6, and 112 mg L^{-1}) showed clear variation in viable cell counts, as illustrated in Figure (6), The control treatment (without herbicide) recorded the highest average logarithmic bacterial count throughout the incubation period, with cells entering the logarithmic phase early and reaching the stationary phase by day six.

Treatment H1 (36 mg/L) exhibited similar behavior to the control during the initial days, followed by a slight decrease in the later days. In contrast, treatments H2 and H3 (52.6 and 112 mg/L) showed a notable delay in entering the logarithmic phase, accompanied by a gradual decrease in cell numbers. This effect was particularly pronounced in treatment H3, which recorded the lowest values throughout the experiment duration.

Statistical analysis revealed significant differences in the average logarithm of bacterial counts among the various treatments on days five and ten, confirming an inverse relationship between herbicide concentration and the number of growing cells. The bacteria passed through all growth phases. Bacterial growth stages were clearly distinguishable in the control treatment (without herbicide addition), moving from the lag phase on day one to the logarithmic phase on days 2-5, where bacterial numbers increased rapidly, followed by the stationary phase on days 6-7. By day 9, a gradual decline in counts was observed, especially in treatment H3, representing the deterioration or death phase. These results indicate the ability of *Paenibacillus alvei* to resist low concentrations of the pesticide, with the inhibitory effect becoming evident at high concentrations. This should be taken into consideration when using it in bioremediation techniques. These results are consistent with what Badaluddin and colleagues reported when they studied the growth curve of isolates of these bacteria under different conditions **(Badaluddin *et al*., 2018).**

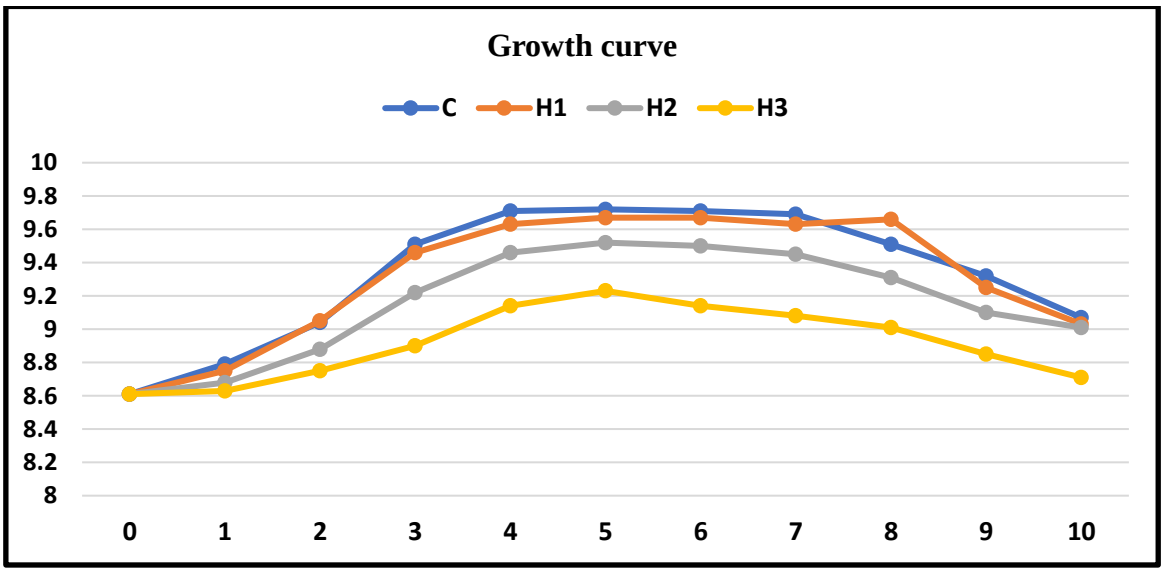

Figure (6) Growth curve of *Paenibacillus alvei*.

Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of *Paenibacillus alvei*

Using agar well diffusion method **(Andrews,2001).** the diameter of the area around the disc (no-growth zone) was 27 and 30 mm at concentration of 62.5 and 125 mg L−1, respectively, which means that there was a noticeable growth cessation of low freezing points in *Paenibacillus alvei*. By comparing the diameters of the inhibition zones among different concentrations and the control treatment (without pesticide), it is evident that pesticide concentrations directly affect bacterial growth. Higher pesticide concentrations resulted in an increased diameter of the inhibition zone, indicating that they fall within the range of the minimum inhibitory concentration (MIC). In contrast, no inhibition zone was observed at concentrations below 62.5 mg L^{-1}. For higher concentrations, the results showed that 250 and 500 mg L^{-1} caused complete bacterial death, as no growth was observed upon subculturing samples onto pesticide-free media, indicating that these concentrations represent the Minimum Bactericidal Concentration (MBC).

Table (5) MIC and MBC ranges for *Paenibacillus alvei.*

Type of Inhibition	Concentration Range (mg L^{-1})	Observations
Non-inhibitory concentration	Less than 62.5	No inhibition zone observed
Inhibitory concentration (MIC)	62.5 – 125	Clear inhibition zones observed
Bactericidal concentration (MBC)	250 – 500	No growth observed upon re-culturing

Our results show that *Paenibacillus alvei* is also resistant to moderate pesticide concentrations but high levels interfere with its biological activities and kill it. This must be considered when it is applied in bioremediation methods. **Grady *et al.* (2016)** showed *Paenibacillus alvei* probably can adopt to environmental circumstances and trigger cellular reactions allowing the bacteria to stand up against the factors suppressing its growth but to a certain degree. US Environmental Protection Agency reports **EPA, (2007)** also indicated that pyroxsulam has potential effects on soil microorganisms, especially at high concentrations, which may be consistent with the results of this study regarding the inhibition of bacterial growth at high concentrations of the pesticide.

Image (1) No inhibition observed at a concentration below 62.5 mg L^{-1}.

Image (2) Inhibitory concentration (MIC) ranging from 62.5 to 125 mg L^{-1}

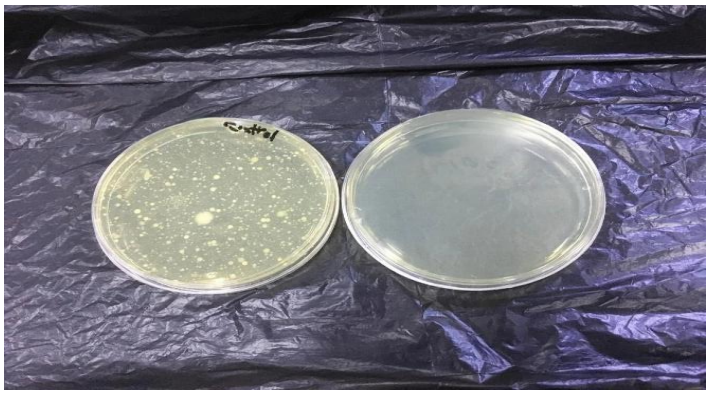

Image (3) Bactericidal concentration (MBC) ranging from 250 to 500 mg L^{-1}.

CONCLUSION

1. The locally isolated *Paenibacillus alvei* from pyroxsulam herbicide-treated soil could grow in a liquoric medium with the herbicide as the only carbon source, which confirms their capacity to metabolize and degrade the pesticide.
2. The results of laboratory study propose the application of this isolate in pesticide bioremediation, especially in agricultural soil with pyroxsulam contamination, as a safe and eco-friendly option.

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