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Genetic Diagnosis of the Bacterial Isolates for Pesticide Analysis of Acetamiprid and Study Optimal Condition for Their Growth

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ABSTRACT

This study aimed to determine the most effective bacterial species for breaking down acetamiprid in soil samples obtained from the greenhouses, 10 bacterial isolates were collected from soil contaminated with pesticides taken from greenhouses of the Horticulture Department/Ministry of Agriculture (Abu Ghraib,Iraq) which has been using these pesticides for a long time on a wide range of agricultural crops such as cucumbers, tomatoes, eggplants and squash. The best bacterial isolates that showed the ability to decompose the pesticide with high efficiency for 21 days are isolates 7, 8, 9, and 10. Isolate No. 9 which had the best biodegradation of the pesticide, was identified and thus genetically diagnosed. The results of the genetic diagnosis of bacteria No. 9 showed that the local bacterial isolate belongs to the genus *Lysinibacillus* of the bacterial strain *Lysinibacillus fusiformis*, which was registered as the first local isolate in the gene bank that worked to degrade or break down the pesticide.The optimum conditions for the growth of the isolated and most efficient degrading bacteria in the decomposition of the pesticide were determined. The optimum temperature for the growth of the strain *Lysinibacillus fusiformis* (PQ109093) was 28 C˚,and the optimum pH for growth was 7.0,and the optimum concentration for the decomposition of the pesticide by bacteria was 25,50 ppm at the concentrations used for the pesticide.

Keywords: acute toxicity,biodegradation, *Lysinibacillus fusiformis*,neonicotinoids.

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INTRODUCTION

The expression "insecticide" refers to any substance utilized independently or in conjunction with other items to prevent, repel, diminish, extract, restrict reproduction, or exterminate pests, It should be remembered that the name is composed of two words: "insect," which means pest, and "cide," which means deadly or killer (Conde-Avila *et al*,2021).The term pesticide has the same meaning, but is more commonly known in the field of agricultural pesticides(Finger, 2024**,** Ajeel and Mohammed,2022).The pesticides are a legitimate tool that can achieve numerous benefits in agriculture, For example The use of pesticides to control agricultural insect pests, pathogens, and weeds avoids the resulting losses and can improve the production and quality of food and staple crops (Aislabie *et al*,2015). Controlling insects of medical and veterinary importance, such as mosquitoes, flies, and other disease-carrying insects, achieves public health benefits. Using poison baits or other suitable preparations to combat rodents helps avoid their problems and health and agricultural damages, as pesticides are an important tool to avoid and treat termite infestations in buildings (Tudi *et al*,2021). Toxic effects resulting from pesticide residues are classified into two types: Acute toxicity – the harmful effect that occurs in an organism after a single, short-term exposure to the pesticide. Sub-acute or chronic toxicity – the harmful effect that occurs in an organism as a result of repeated exposure to the pesticide for a period longer than half of the organism's lifespan **(**Nayak and Solanki,2021**,** Poudel *et al*,2020**).**Chronic toxicity leads to risks

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resulting from continuous exposure to low concentrations of pesticides, which results in residues of those pesticides that have a harmful effect on the body for long periods of time, causing various types of diseases, the most important of which is cancer (Zhou *et al*,2024**,** Sandoval-Insausti *et al*,2021). One of the most important toxic effects of those pesticides is the effect on the nervous system, through inhibiting the enzyme cholinesterase, as acetylcholine is a very important substance in transmitting nerve impulses (Ali *et al*,2021**,** Oyugi *et al*,2021).The use of microorganisms is one of the most important strategies for removing pesticides through enzymatic biodegradation, this process is more effective, less dangerous, and economical, in addition to being environmentally friendly(Balasim *et al*,2024). Moreover, biodegradation is not limited to just getting rid of pollutants (pesticide residues), Therefore these organisms that carry out the biodegradation process can be used in biofertilization, so they can be added as fertilizers to the soil, which have many activities in enhancing plant growth, such as the production of plant growth hormones, nitrogen fixation, dissolving phosphate, hydrogen cyanide and iron carriers (Jacquet *et al*,2022, Verma *et al*,2014**,** Mohammed and Hasan,2023).Since these pesticides give some microorganisms an adequate supply of energy, carbon, nitrogen, and electrons, they are regarded as a bioremediation method in terms of their ability to break down pesticides and, consequently, purify areas contaminated by pesticides and their residues (Jasim and Hamid,2023).Another study (Ray *et al*,2024) stated that it is not necessary that only the microorganisms present in the soil are the ones that decompose pesticides, as there are many groups of microorganisms that decompose these pesticides, including organisms that were isolated from an environment in which these substances (pesticides) are present(Ashraf *et al*,2023**,** Mohammed *et al*,2023).These organisms are distinguished by their ability to live in extreme environmental conditions because they tolerate or love the presence of these materials and analyze them according to specific mechanisms or special enzymes that are responsible for the decomposition of these materials and being aware of the ideal growing conditions such as temperature, pH, and other factors for these minuscule organisms that break down pesticides (Wang ***et al*,2024,** Asira, 2013**,** Chen *et al*,2021).This study aimed to determine the most effective bacterial species for breaking down acetamiprid in soil samples obtained from the greenhouses and Study the optimal conditions for the growth of pesticide-degrading bacteria.

MATERIALS AND METHODS

Chemicals and instruments:

The acetamiprid chemical pesticides (purity > 99.6%) has been provided by the National Centre for pesticide Control's Plant Protection Department. We purchased the 99.9% pure HPLC-grade solvents from Sykam in Germany. Acetonitrile was used along with HPLC-grade water and the Millipore filter (0.45 µm). Aliquots of the working standard solution for HPLC calibration were used to establish analytical standards. The range ranged between 1- 100 mg/L.An HPLC (model S2300 Sykam, Germany) was used for the analysis of acetamiprid. Clarity Station software was used for all data collection and processing.

Gathering samples of soil

soil samples were gathered at randomly from greenhouses that were owned by the Ministry of Agriculture's Horticulture Department in the Abu Ghraib-Iraq. The samples were taken from the 50 m^2 rhizosphere area, reaching a depth of 15–30 cm. They were then put in sterile nylon bags, and then taken to the lab for more research.

Pesticide-degrading bacteria isolation: According to (Gomaa *et al*,2020) 2.7 g of dry soil was added with 25 ml of sterilized Mineral Salt Medium (MSM) broth {this medium was prepared as the following components were added: monosodium phosphate NaH_2PO_4 (1.0 g/L), potassium hydrogen phosphateK_2HPO_4 (0.625 g/L), magnesium sulfate $MgSO_4$ (0.2 g/L), Ammonium nitrate NH_4NO_3 (1.0 g/L), Disodium hydrogen phosphate Na_2HPO_4 (1.0 g/L), Monopotassium phosphate KH_2PO_4(0.5 g/L) and sodium chloride NaCl (1.0 g/L, in one liter of distilled water and the pH was adjusted to 7.0} to the flask 100 ml. Then it

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was put in a rotary shaker for 2 hours at a speed of 220 rpm, after that being taken 5 ml to a flask 500 ml that contain 200 ml of MSM with 25 ppm acetamiprid using a Millipore filter 0.22 µm at pH 7.0, then incubated for one week at 28 C˚ in a rotary shaker at 120 rpm. By distributing a 0.1 ml aliquot of culture MSM agar plate with 25 ppm acetamiprid and incubating it for 24-72 hours,bacterial colonies resistant to acetamiprid were found. The colonies of bacteria in the plates that stood out from the others were then located. To acquire isolated substances that are pure Re-culturing a few isolated colonies on Nutrient Agar (N.A) {this medium was prepared according to the instructions of the supplier company (HiMedia, India)} and incubating them for 24–72 hours at 28 C˚.

Bioderation of pesticide by bacteria isolates

Sterilized flask 50 ml were used for the biodegradation of pesticides in (MSM). Each flask had 30 ml of MSM with 25 ppm acetamiprid added. The flasks were inoculated with bacterial cells 10^7CFU/ml for a total of three weeks at 28C˚ in the dark on a rotary shaker set to 120 rpm in order to decompose the acetamiprid. Control flasks, which contained medium (MSM) and the insecticide acetamiprid 25 ppm (no bacteria), were also utilized. For pesticide residue investigations, subsamples were taken at 1, 7, 15,and 21 days from each treatment. A half milliliter of each subsample and a half milliliter of acetonitrile were combined in two milliliter eppendorf tubes in order to ascertain the concentration of each treatment. After centrifuging the mixture for 10 min at 12,000 rpm, the supernatant was transferred to amber HPLC vials using Pasteur pipettes and then placed in the refrigerator. The HPLC was filled with 50 µl of each sample.

Molecular Characterization

The bacterial isolate was genetically identified as more efficient than other bacterial isolates in degrading acetamiprid by 16S ribosomal DNA.The National Centre for Biotechnology Information's (NCBI) rRNA database was used for sequence analysis and homogenic data verification after bacterial ribosomal RNA amplification ,The primers used in this study was 27F(Primer Name) with this Sequence: 5`-AGAGTTTGATCCTGGCTCAG-3`, 1492R (Primer Name) with this Sequence: 5`-TACGGTTACCTTGTTACGACTT-3`and the Annealing Temperature was 60 C˚, Product size was 1500 bp. the Primer Preparation was from The Macrogen Company its supplied the primers in a lyophilized Quantus Fluorometer and After dissolving lyophilized primers in water with no nuclease, a stock solution was created with a final concentration of 100 pmol/µl , primer stock solution(stored at -20C˚) was mixed with 90µl of nuclease-free water(Sulaiman *et al*, 2018).

DNA extraction: The extraction of DNA protocol was assessed by taking 1 ml overnight culture was spun at 13,000 rpm to pellet the cells. The pellet was resuspended in Buffer BL with Proteinase K and incubated at 56C˚. Ethanol was added, and then several washes were performed with Buffer BW and TW using a mini-column. After a final centrifugation step, DNA was finally eluted using Buffer AE.

PCR Reaction Mix Preparation and PCR Thermocycling Conditions: PCR assays were conducted at a reaction volume of 50 µl.Taq Ready master mix kit was used for PCR reaction and the equipment was from a company Promega,USA.

The sample preparation and using Gel Electrophoresis: Agarose gel electrophoresis was conducted to verify the presence of DNA after PCR amplification.An agarose gel 1.5% was made with TAE buffer and Ethidium Bromide for staining purposes.The gel was introduced into the tray for electrophoresis, set at ambient temperature.Electrophoresis was performed at 100V for 60 minutes after loading the PCR products.DNA bands were observed with a gel imaging system, and sequencing was performed by Macrogen using Geneious software.

Studies on growth kinetics (optimization of bacteria growth):

The effect of temperatures: MSM broth was adjusted to 7.0, autoclaved and sterilized Pesticide was added, and flasks were inoculated with a purified bacterial isolate 10^7CFU/ml, Incubated at various temperatures, microbial growth was assessed by measuring

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absorbance at 550 nm. A graph was created using the average absorbance value. (Mehta *et al*, 2021).

The effect of (pH) levels:

MSM broth was sterilized and adjusted to the ideal pH for microbial growth. A sterilized pesticide was applied, and purified bacteria were inoculated, the flasks were incubated at 28 C˚ .The ability of microbial growth was assessed by measuring absorbance at 550 nm after a period of 1, 4, 7, and a graph was created (Parit *et al*,2018).

The effect of Agitation Speed

MSM broth was prepared, sterilized, and the pH was adjusted, The sterilized pesticide was added to it ,The flasks were inoculated with the bacterial isolate and incubated in a shaking incubator at different stirring speeds at the 28 C˚. after 1, 4, 7, 10 and 14days of incubation and stirring, The light absorption value was measured at a wavelength of 550 nm to determine the effect of stirring speed on the development of the growth of the pesticide-degrading bacterial isolate,and a graph was created**(**Gangola *et al*,2024**).**

The effect of acetamiprid Concentrations

MSM broth was made and sterilized ,The different concentrations of acetamiprid was was added aseptically to the medium, the flasks were inoculated with a purified bacterial, the flasks were incubated at 28 C˚and pH 7.0 after 1,4,7,10,14,and 21 days Following incubation, the absorbance at 550 nm was measured to determine the development of isolates that breakdown pesticides. a graph was created (Sahoo *et al*,2024).

Statistical analysis

This study used SPSS to analyze HPLC results revealing variations in acetamiprid concentration across treatments,comparing means using LSD-Least Significant Difference (ANOVA-one way)

RESULTS AND DISCUSSION

Residues of pesticides detected through HPLC for bacterial isolates that degrade pesticides:

Before any sample analyses were injected, the HPLC was calibrated using working standard solutions of acetamiprid at different dilutions 1, 10, 25, 50,and 100 ppm, in order to determine its sensitivity. The correlation between the amount of standard solution injected and the resulting peak area for acetamiprid was 0.99, indicating a linear relationship and the Pesticide residues were determined by using HPLC for bacterial isolates from soil. Various bacterial isolates were employed to assess the biodegradation of acetamiprid over a period of 21 days. A one-way ANOVA was utilized to investigate differences among ten isolates obtained from (MSM) in the soil samples, the One-way analysis of variance (ANOVA) is employed when there is one independent variable, and it is used to determine if there are any statistically significant differences among the treatments. the L.S.D. values for 1, 7, 14,and 21 days were 0.109, 0.128, 0.106, 0.137, indicating that means with different letters in the same column differed significantly, * ($P \leq 0.05$) through statistical analysis (table1).The isolates No. 9 excelled for its capacity to reduce the pesticide level by 70% In comparison to other bacterial isolates, which varied in their capacity to break down the pesticide (**15**).the acetamiprid concentration measured by HPLC for isolates No. 9 grown in MSM medium for 21 days was 13.6 ppm, while for isolates No. 10 it was 15.7 ppm. Isolates No. 7 and 8, HPLC estimates of their acetamiprid concentrations being 19.2 and 20.11 ppm (Figure1).through the results obtained, it was found that isolate No.9 surpassedhe other isolates in its efficiency in decomposing the pesticide and using it as a source of carbon and energy necessary for growth. Bacterial isolates' biodegradation of pesticides varies based on the type of microorganism, aligning with the concept of a group of microorganisms capable of this process (Guo *et al*, 2021).

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Table1. Residue of acetamiprid (ppm) by unidentified bacterial isolates in (MSM) at 21 days was assessed using HPLC

Residue of acetamiprid ppm/days				
Isolation number	**1 day**	**7days**	**14days**	**21days**
Control	40.53 ±0.02 a	40.07 ±0.03 a	35.21 ±0.02 b	30.51 ±0.02 a
1	34.37 ±0.06 g	30.69 ±0.03 e	31.72 ±0.08 c	30.21 ±0.01 b
2	30.32 ±0.01 i	30.84 ±0.03 d	28.83 ±0.02 e	26.23 ±0.01 d
3	35.37 ±0.08 e	33.20 ±0.06 c	30.53 ±0.03 d	28.34 ±0.03 c
4	38.52 ±0.01 c	35.28 ±0.04 b	39.52 ±0.01 a	30.24 ±0.09 b
5	40.38 ±0.02 b	40.18 ±0.07 a	30.46 ±0.03 d	25.20 ±0.09 e
6	40.53 ±0.02 a	35.16 ±0.03 b	30.43 ±0.03 d	20.43 ±0.03 f
7	35.16 ±0.03 f	30.20 ±0.06 f	22.48 ±0.02 f	19.16 ±0.03 h
8	35.36 ±0.03 e	30.57 ±0.04 e	22.43 ±0.04 f	20.14 ±0.03 g
9	30.63 ±0.03 h	25.83 ±0.03 g	19.26 ±0.01 h	13.62 ±0.01 j
10	35.82 ±0.01 d	30.63 ±0.01 e	19.92 ±0.01 g	15.70 ±0.02 i
L.S.D. value	0.109 *	0.128 *	0.106 *	0.137 *
Means having with the different letters in same column differed significantly * (P≤0.05)				

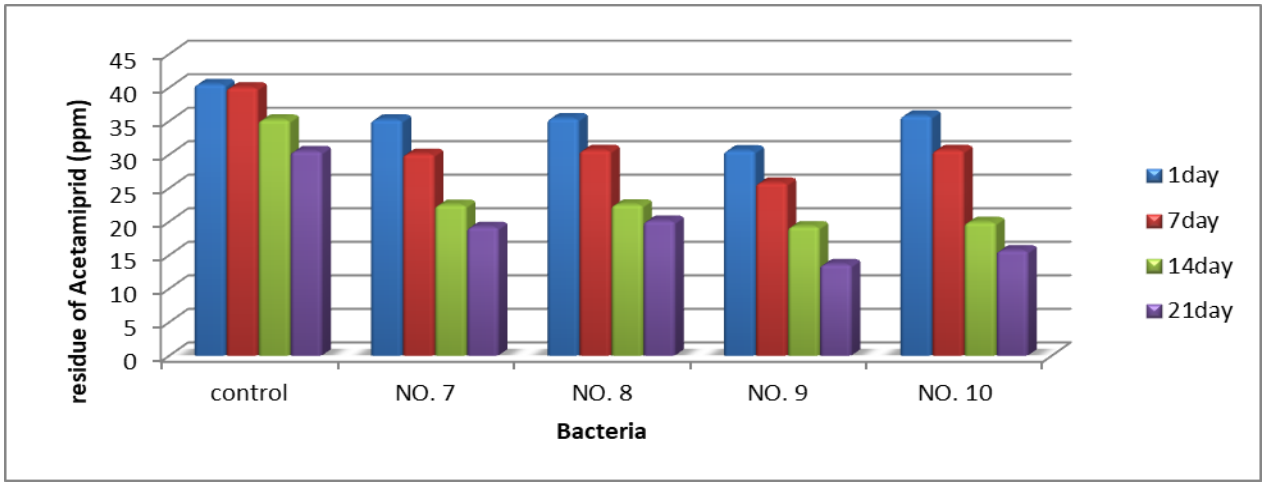

Figure1. Impact of four isolated bacteria on the degradation of the pesticide acetamiprid in MSM

Molecular Characterization of acetamiprid-degrading bacteria No. 9: The selected isolates were identified at the molecular level by analyzing the sequence of the 16S rRNA gene. The 16S rRNA sequences of the isolates were compared with similar genomes of other bacteria found in GenBank using ntBlast and other methods. According to the PCR data (Figure.2),a band measuring (1500) bp was found in the duplicated isolate. The 16S rRNA segment was found to have a BLASTN comparison with existing bacterial sequences in the GenBank database, revealing a 100% sequence similarity to nitrogenous bases of *Lysinibacillus fusiformis*. This sequence is recorded in GenBank (NCBI) under the accession numbers (PQ109093).

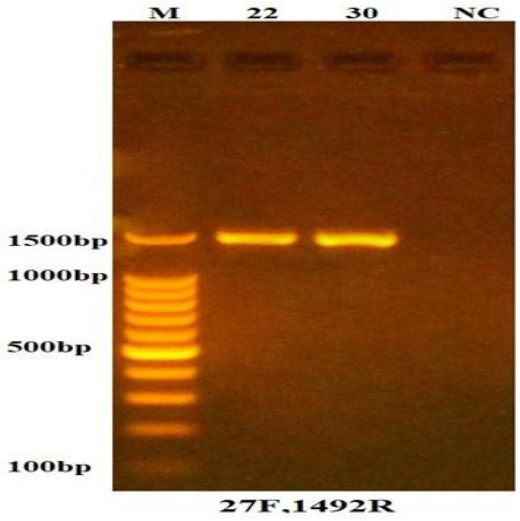

Figure 2. Results of the amplification of 16s RNA gene of *Lysinibacillus fusiformis*. On gel electrophoresis,the symbol (M) represents the volume index (ladder) with a molecular weight ranging from 100-1500 pb

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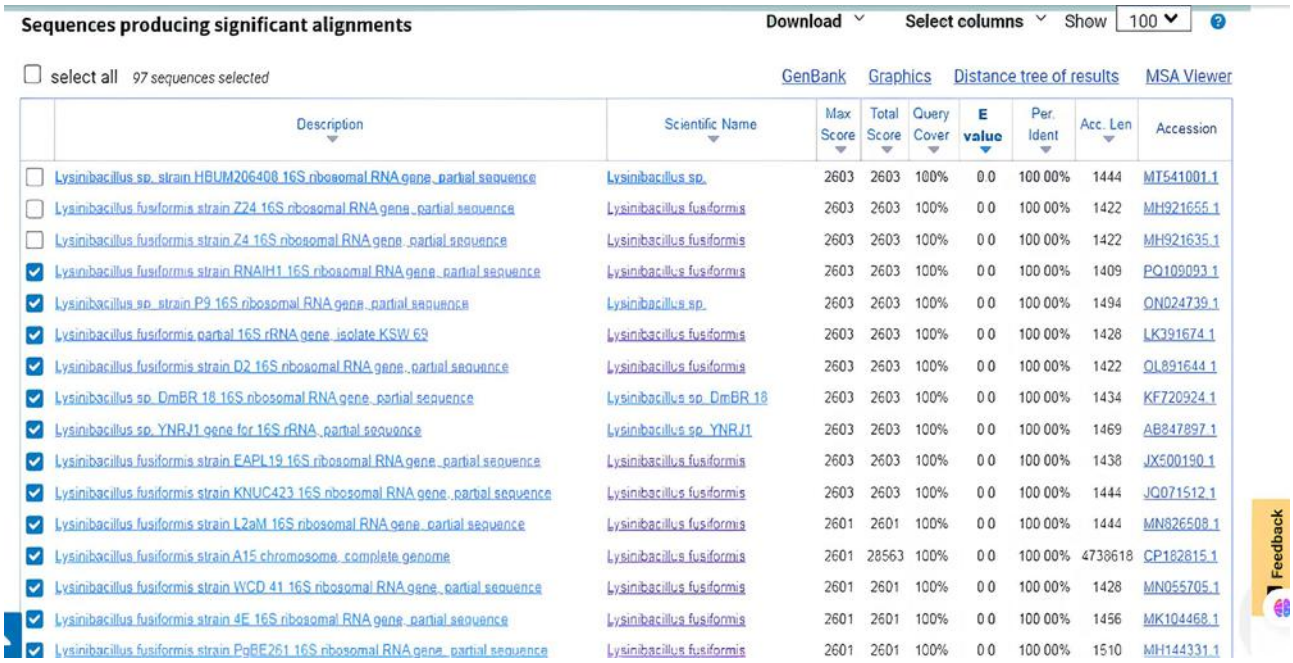


Sequences producing significant alignments — Download — Select columns — Show 100

select all 97 sequences selected — GenBank — Graphics — Distance tree of results — MSA Viewer

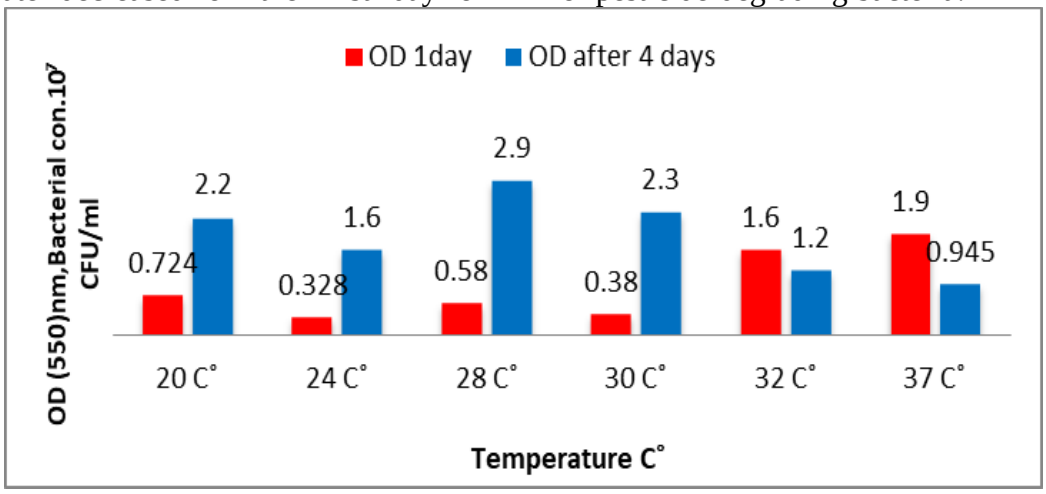
Description	Scientific Name	Max Score	Total Score	Query Cover	E value	Per. Ident	Acc. Len	Accession
Lysinibacillus sp. strain HBUM206408 16S ribosomal RNA gene, partial sequence	Lysinibacillus sp.	2603	2603	100%	0.0	100.00%	1444	MT541001.1
Lysinibacillus fusiformis strain Z24 16S ribosomal RNA gene, partial sequence	Lysinibacillus fusiformis	2603	2603	100%	0.0	100.00%	1422	MH921655.1
Lysinibacillus fusiformis strain Z4 16S ribosomal RNA gene, partial sequence	Lysinibacillus fusiformis	2603	2603	100%	0.0	100.00%	1422	MH921635.1
Lysinibacillus fusiformis strain RNAIH1 16S ribosomal RNA gene, partial sequence	Lysinibacillus fusiformis	2603	2603	100%	0.0	100.00%	1409	PQ105093.1
Lysinibacillus sp. strain P9 16S ribosomal RNA gene, partial sequence	Lysinibacillus sp.	2603	2603	100%	0.0	100.00%	1494	ON024739.1
Lysinibacillus fusiformis partial 16S rRNA gene, isolate KSW 69	Lysinibacillus fusiformis	2603	2603	100%	0.0	100.00%	1428	LK391674.1
Lysinibacillus fusiformis strain D2 16S ribosomal RNA gene, partial sequence	Lysinibacillus fusiformis	2603	2603	100%	0.0	100.00%	1422	OL891644.1
Lysinibacillus sp. DmBR 18 16S ribosomal RNA gene, partial sequence	Lysinibacillus sp. DmBR 18	2603	2603	100%	0.0	100.00%	1434	KF720924.1
Lysinibacillus sp. YNRJ1 gene for 16S rRNA, partial sequence	Lysinibacillus sp. YNRJ1	2603	2603	100%	0.0	100.00%	1469	AB847897.1
Lysinibacillus fusiformis strain EAPL19 16S ribosomal RNA gene, partial sequence	Lysinibacillus fusiformis	2603	2603	100%	0.0	100.00%	1438	JX500190.1
Lysinibacillus fusiformis strain KNUC423 16S ribosomal RNA gene, partial sequence	Lysinibacillus fusiformis	2603	2603	100%	0.0	100.00%	1444	JQ071512.1
Lysinibacillus fusiformis strain L2aM 16S ribosomal RNA gene, partial sequence	Lysinibacillus fusiformis	2601	2601	100%	0.0	100.00%	1444	MN826508.1
Lysinibacillus fusiformis strain A15 chromosome, complete genome	Lysinibacillus fusiformis	2601	28563	100%	0.0	100.00%	4738618	CP182815.1
Lysinibacillus fusiformis strain WCD 41 16S ribosomal RNA gene, partial sequence	Lysinibacillus fusiformis	2601	2601	100%	0.0	100.00%	1428	MN055705.1
Lysinibacillus fusiformis strain 4E 16S ribosomal RNA gene, partial sequence	Lysinibacillus fusiformis	2601	2601	100%	0.0	100.00%	1456	MK104468.1
Lysinibacillus fusiformis strain PgBE261 16S ribosomal RNA gene, partial sequence	Lysinibacillus fusiformis	2601	2601	100%	0.0	100.00%	1510	MH144331.1

Feedback

Figuer 3. BLAST results isolate No.9 16S rRNA gene, partial sequence in NCBI

Optimal conditions for bacterial isolates growth (*Lysinibacillus fusiformis*)

Optimal temperature: The effect of temperature on the growth rate of *Lysinibacillus fusiformis* bacteria was determined by using a range of temperatures, The optimum temperature for bacterial growth was 28C˚ after four days of incubation,The best bacterial growth can be achieved after 4 days of incubation so the results were taken on the first day and after the fourth day. In the same applies to a temperature of 30C˚, which was ideal for bacterial growth, While the growth rate decreased on the first day of incubation with the increase in temperature from 32-37C˚,as in (Figure.4), and this is consistent with (**10**) regarding the difference in temperatures affecting the biodegradation of the pesticide acetaimprid ,As he stated (**36,38**), bacterial growth occurs over a broad temperature range of 20-37C˚,which is consistent with this information.*Ochrobactrum sp*. had an optimum growth temperature of 20-35C˚, while *Pigmentiphaga sp.* thrived at 30-42C˚.Both genera are part of the same group of pesticide-degrading bacteria.



Figuer 4. Optimal Temperature of *Lysinibacillus fusiformis* in 1 day, after 4 days

Optimal pH: The optimum pH for the growth of *Lysinibacillus fusiformis* bacteria was 7.0 within the specified range 4.0-7.0, When the test conditions for this experiment are established (1,4days).It was observed that growth was limited at acidic pH 4.0, while growth was normal at neutral and alkaline pH during different time periods(figer.5).This

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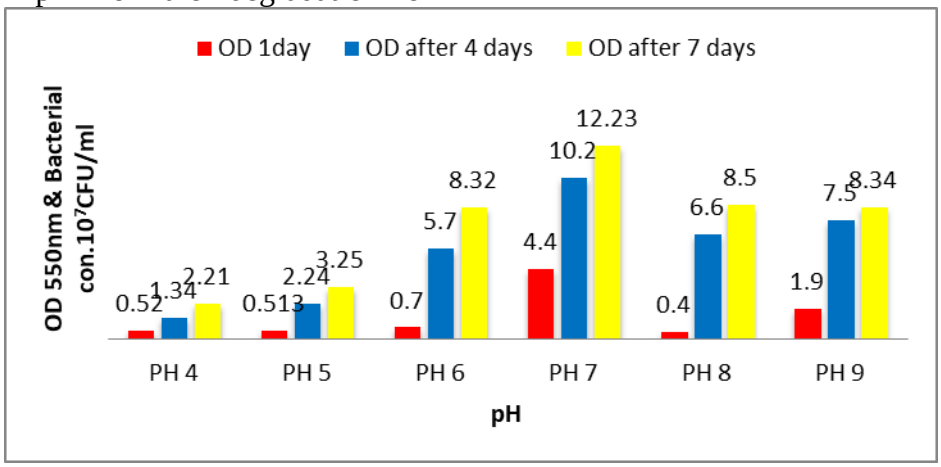
result was consistent with what was mentioned by (Wang *et al*,2024) in the test of the optimum pH number for bacterial growth like *Lysinibacillus sp, Pseudoxanthomonas sp.,Bacillus sp* it was found to be 7.0, which is the optimum pH for the degradation of pesticides by these bacteria. This resultat showed a strong correlation with the findings of report (Mehta *et al*,2021),which aimed to identify the best pH for biodegrading of pesticides by isolate *Lysinibacillus fusiformis.*



Figuer.5 Optimal pH of *Lysinibacillus fusiformis* in (1) day, after (4) days, after (7) days

Optimal Agitation Speed: The agitation speed had a significant effect on the growth of pesticide-degrading bacteria, as the optimal growth of pesticide-degrading bacteria in the shaking incubator was at a speed of 150, 200 rpm for 7 days of incubation and when using different stirring speeds 50, 100, 150 ,200,and 250 rpm at the optimal temperature and optimal pH for 14 days of incubation.This result is consistent with (Hasan and Shakir,2025) which indicated that when isolating pesticide-degrading bacteria, a stirring speed higher than 120 rpm must be provided and when isolating this type of bacteria, the agitation speed should be 180-220 rpm.It was noted that the growth rate was low at agitation speeds of 50 and 100, and from the first day of incubation,As shown in (Figure.6).Using excessive speed has an effect on the cells, as it causes them to break down, while slow speed leads to the cells clumping together, thus reducing the contact between the cells and the nutrients (Ghanbarpour and Ghanbarpour, 2024).

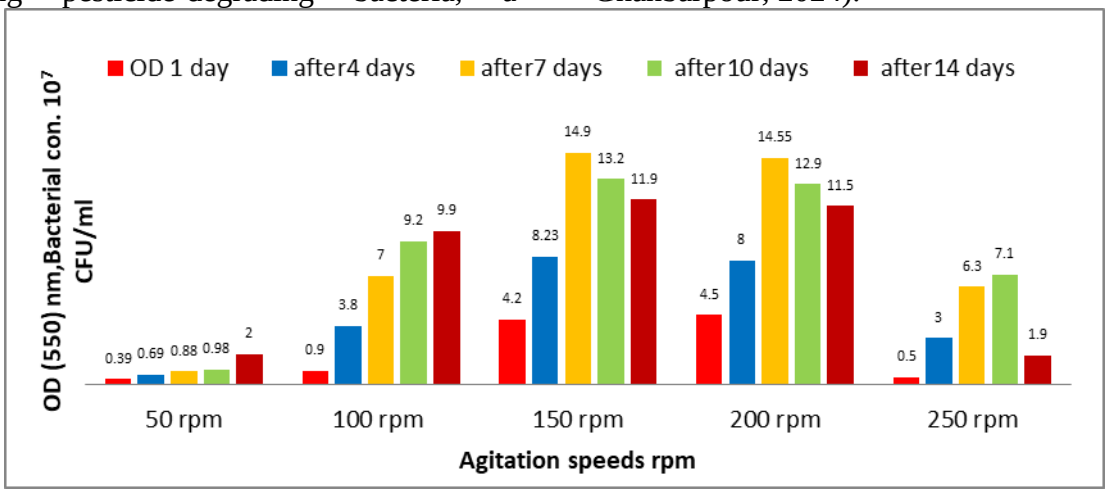


Figure 6. Optimal agitation speed for *Lysinibacillus fusiformis* during 1,4,7,10,and14 days

Optimal acetamiprid concentration

The best concentration of pesticide for decomposition by *Lysinibacillus fusiformis* bacterium within the specified range 25-100 ppm was (25,50ppm) at the optimal temperature and optimal pH for 21 days of incubation , This result consistent with that mentioned in (Datta *et al*,2024**,** Zhang *et al*,2024) that the best concentration of pesticide for decomposition by bacteria was

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25ppm. Thus, biodegradation becomes less the higher the concentration of pesticide, as shown in the (figure.7), the incubation period had a noticeable effect on bacterial growth at a concentration of 50ppm of the pesticide, especially after 7 days of incubation, as the increase in growth was slight compared to the increase at a concentration of 50ppm.

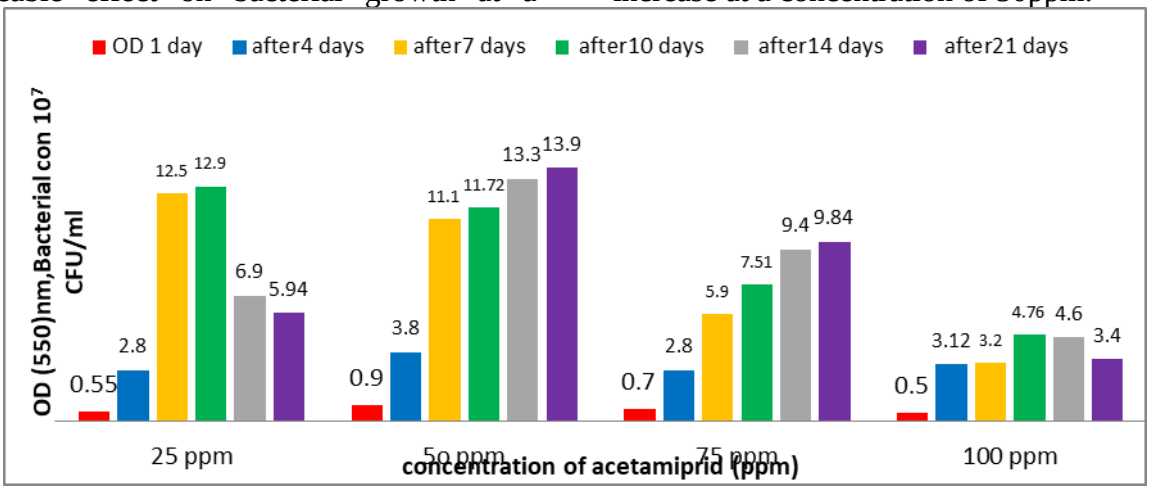

Figure 7. Optimal concentration of acetamiprid for *Lysinibacillus fusiformis* during 1,4,7,10,14,and 21 days

CONCLUSION

This study concluded that are many types of bacterial isolates that can be used to degrade pesticides, especially for the pesticide acetamparatide. Among them is the bacterium *Lysinibacillus fusiformis*, which is the best new bacterial isolate that can be used for biodegradation of pesticides.

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عزل وتشخيص البكتريا المحللة للمبيد الحشري Acetamiprid ودراسة الظروف المثلى لنموها

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المستخلص

هدفت هذه الدراسة إلى تحديد أكثر أنواع البكتيريا فعالية في تحليل مبيد الأسيتامبريد في عينات التربة المأخوذة من البيوت البلاستيكية,جمعت 10 عزلات بكتيرية من التربة الملوثة بالمبيد المأخوذة من البيوت البلاستيكية التابعة لدائرة البستنة/وزارة الزراعة (ابو غريب,العراق)والتي تستخدم هذه المبيدات لفترة طويلة على مجموعة واسعة من المحاصيل الزراعية مثل الخيار والطماطم والباذنجان والقرع.أفضل العزلات البكتيرية التي أظهرت القدرة على تحليل المبيد بكفاءة عالية لمدة 21 يومًا هي العزلات 7و8 و9 و10.تم تحديد العزلة رقم 9،والتي كان لها أفضل تحلل حيوي للمبيد،وبالتالي تم تشخيصها وراثيًا.أظهرت نتائج التشخيص الجيني للبكتيريا رقم 9 أن العزلة البكتيرية المحلية تنتمي إلى جنس *Lysinibacillus* من سلالة البكتيريا *Lysinibacillus fusiformis* والتي سجلت كأول عزلة محلية في بنك الجينات عملت على تحليل أو تفكيك وتكسير المبيد.وتم تحديد الظروف المثلى لنمو البكتريا المحللة المعزولة والاكفأ في تحليل المبيد فكانت درجة الحرارة المثلى لنمو سلالة *Lysinibacillus fusiformis* (PQ109093) هي 28 م°,والرقم الهيدروجيني الامثل للنمو هي 7.0،وكان التركيز الأمثل لتحلل المبيد بوساطة البكتريا هو 25، 50 جزء في المليون عند التراكيز المستعملة للمبيد.

الكلمات المفتاحية: السمية الحادة، التحلل الحيوي، *Lysinibacillus fusiformis* ، النيونيكوتينويدات.