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Evaluating the bioremediation potential of marine bacteria: Chlorpyrifos tolerance, degradation efficiency and enzyme production

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ABSTRACT

Chlorpyrifos-degrading bacterial isolates were screened for growth tolerance, degradation efficiency, time-dependent degradation and extracellular enzyme production. Among the 31 isolates tested, only a limited subset tolerated and utilized chlorpyrifos effectively, with *(B. amyloliquefaciens, B. cereus, B. subtilis, Enterobacter* sp., *P. aeruginosa* and *P. mendocina)* showing growth at all tested concentrations, indicating strong resistance and metabolic adaptation. Quantitative degradation assays revealed that *Enterobacter* sp. and *P. aeruginosa* exhibited the highest chlorpyrifos degradation at 25 µL, while AKCD19 consistently remained the best performer across 50, 75 and 100 µL concentrations, followed by *(B. cereus, B. subtilis, Enterobacter* sp., *P. aeruginosa* and *P. mendocina)*. Spectrophotometric analysis over 10 days confirmed a progressive increase in degradation, with AKCD19 reaching the highest level of chlorpyrifos breakdown by the end of incubation. Extracellular enzyme profiling showed that the efficient degraders produced one or more hydrolytic enzymes, suggesting a possible role of these enzymes in chlorpyrifos metabolism. *P. aeruginosa* demonstrated the broadest and highest enzyme activity, followed by *B. subtilis*, *P.mendocina*, *B. cereus* and *Enterobacter* sp. Overall, the results indicated a positive association between chlorpyrifos tolerance, degradation potential and extracellular enzyme production, identifying *P. aeruginosa* as the most promising isolate for chlorpyrifos bioremediation.

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INTRODUCTION

The unrestrained application of chemical pesticides, herbicides and fumigants by the agricultural sector for managing pests and weeds has sparked significant apprehension regarding their harmful impacts on both the environment and human health. Reports highlighting this concern are substantiated by the observation that there is a 1.8% rise in pesticide usage per hectare for every 1% increase in crop yield per hectare (Nion and Toyota, 2015; Khalili *et al.*, 2019). In agricultural settings, over 500 distinct pesticide formulations are employed, the majority of which are highly toxic, persistent, non-biodegradable and remain in the soil (Douglas *et al.*, 2018). The situation is further aggravated by the global proliferation of large-scale industries, which contribute to the naturally occurring halogenated compounds. Additionally, effluents from pharmaceutical, petrochemical and agrochemical industries that manufacture pesticides, herbicides and polyhydric compounds contain elevated levels of organic, inorganic and nitrogenous substances, along with dissolved solids (Lu *et al.*, 2014). These include compounds such as 2,2-dichloropropionic acid (2,2-DCP), 2,3-dichloropropionic acid (2,3-DCP), d,l-2-chloropropionic acid (d,l-2CP), 3-chloropropionic acid (3CP), haloacetates like monochloroacetate (MCA), dichloroacetate (DCA), trichloroacetate (TCA) and organophosphates (Lefebvre and Moletta, 2006). The improper disposal of such toxic pollutants poses a significant threat to both ecological systems and human health (Lu *et al.*, 2014; Aloui *et al.*, 2009). Agricultural runoff and industrial discharges ultimately flow into water bodies, such as rivers, before reaching the oceans (Nemati *et al.*, 2013; Edbeib *et al.*, 2017). Over time, these hazardous substances accumulate in marine organisms and are subsequently consumed by birds and mammals higher up the food chain (Khalili *et al.*, 2019; Abdulgader Edbeib *et al.*, 2016; Abidin *et al.*, 2019). This aligns with findings from the World Health Organization which attributes environmental pollution as a contributing factor to 1.7 million child deaths (Abidin *et al.*, 2019).

The exposure to pesticides and instances of poisoning are inflicting considerable harm on flora, fauna and human health (Jaacks *et al.*, 2019). The presence of pesticides has been identified through the analysis of their residual concentrations in water, soil and air, posing threats to biological systems and human well-being via the food chain (Schleifer and Speiser 2022; Chaudhari *et al.*, 2023). Pesticides have polluted rivers, lakes and adjacent agricultural regions, leading to elevated pesticide levels in aquatic ecosystems (Sruthi *et al.*, 2017).

Humans encounter pesticides such as hexachlorocyclohexane and dichloro diphenyl trichloroethane through the consumption of organisms from contaminated aquatic environments, which may lead to various health issues, including endocrine disruptions, birth defects, prostate and uterine cancers and diminished sperm counts (Jayaraj *et al.*, 2016).

The unique characteristics of hypersaline environments such as those found in Lake Tuz, lead to an increased diversity of microbial life (halophiles) that inhabit these areas, necessitating their specialized adaptation into salt-loving microbiomes. This category of microbes possesses the ability to withstand the physical stress associated with high salinity, thrive and perform optimal metabolic activities (Le Borgne *et al.*, 2008), thereby making them promising candidates for the treatment of wastewater generated by coastal aquaculture sectors (Vymazal and Brezinova, 2014). The elevated salt concentrations in effluents from these industries disrupt the functionality of wastewater treatment facilities, rendering traditional biodegradation techniques ineffective (Lu *et al.*, 2014; Aloui *et al.*, 2009). Consequently, the exploration of efficient salt-tolerant microbes in marine and hypersaline ecosystems that can degrade halogenated pollutants *in situ* may be beneficial in addressing these challenges.

Among the most prevalent bacterial taxa identified in these environments are aerobic, Gram-positive members of the *Bacillaceae* family, particularly the genus *Bacillus* (Gontang *et al.*, 2007).

Microorganisms play an essential role in the degradation of organic pollutants. Numerous studies have documented certain bacteria capable of breaking down chemical contaminants.

Although their degradation pathways have been thoroughly examined, evidence regarding the microbial degradation of organophosphates in shallow groundwater within coastal communities remains scarce. The minimal research on Chlorpyrifos-degrading bacteria in shallow groundwater. The majority of studies have focused primarily on bacteria such as *Alcaligenes faecalis* (Yang *et al.,* 2005), *Bacillus fumilis* (Li *et al.* 2008) and *Pseudomonas aeruginosa* (Vidya Lakshmi *et al.* 2009), which are known to degrade Chlorpyrifos pesticides.

MATERIALS AND METHODS

Isolation and maintenance of bacterial isolates

A bacterial isolates were obtained from environmental marine water samples were collected from Kollam, Calicut and Kochi (Table 1). Isolates were initially enriched in Mineral Salt Medium (MSM) supplemented with chlorpyrifos (20 mg L^{-1}) as the sole carbon source and incubated at 30 °C on a rotary shaker at 150 rpm for 7 days. Pure cultures were obtained by serial dilution and spread plating on MSM agar containing chlorpyrifos followed by sub-culturing on nutrient agar for maintenance. Cultures were stored at 4 °C and sub-cultured for experimental use (Rayu *et al.,* 2017).

Growth tolerance screening to chlorpyrifos

Growth tolerance of bacterial isolates was assessed in MSM broth supplemented with increasing concentrations of chlorpyrifos (25, 50, 75 and 100 µL mL^{-1}). A 24-hour-old bacterial culture was adjusted to an optical density of 0.8 at 600 nm (OD_{600}) and 1 mL of this suspension was inoculated into 100 mL of MSM containing the respective chlorpyrifos concentration. Inoculated flasks were incubated at 30 °C on a rotary shaker at 150 rpm for 7 days. Growth was monitored visually by turbidity and confirmed by measuring OD at 600 nm in the intervals 24-hour using a UV-Vis spectrophotometer. An uninoculated MSM flask with chlorpyrifos served as a negative control. Isolates showing visible growth at each concentration were recorded as tolerant (Yang *et al.,* 2005).

Table 1. The bacteria isolated from the marine water sample

Code	Name of the bacteria
AKCD01	*Acinetobacter* sp.
AKCD02	*Alteromonas macleodii*
AKCD03	*Aneurinibacillus aneurinilyticus*
AKCD04	*Bacillus albus*
AKCD05	*Bacillus altitudinis*
AKCD06	*Bacillus amyloliquefaciens*
AKCD07	*Bacillus cereus*
AKCD08	*Bacillus haynesii*
AKCD09	*Bacillus licheniformis*
AKCD10	*Bacillus subtilis*
AKCD11	*Bacillus tequilensis*
AKCD12	*Enterobacter* sp.
AKCD13	*Halomonas* sp.
AKCD14	*Marinobacter hydrocarbonoclasticus*
AKCD15	*Micrococcus luteus*
AKCD16	*Photobacterium damselae*
AKCD17	*Planococcus* sp.
AKCD18	*Pseudoalteromonas sp.*
AKCD19	*Pseudomonas aeruginosa*
AKCD20	*Pseudomonas mendocina*
AKCD21	*Serratia marcescens*
AKCD22	*Serratia* sp.
AKCD23	*Shewanella bicestrii*
AKCD24	*Shewanella putrefaciens*
AKCD25	*Shewanella seohaensis*
AKCD26	*Staphylococcus arlettae*
AKCD27	*Staphylococcus cohnii*
AKCD28	*Staphylococcus saprophyticus*
AKCD29	*Vibrio alginolyticus*
AKCD30	*Vibrio harveyi*
AKCD31	*Vibrio parahaemolyticus*

Quantitative chlorpyrifos degradation assay

The quantitative degradation potential of isolates was evaluated in MSM broth supplemented with chlorpyrifos at concentrations of 25, 50, 75 and 100 µL L^{-1}. Each 250 mL flask containing 100 mL of MSM with chlorpyrifos was inoculated with 1 mL of 24-hour-old bacterial culture. Flasks were incubated at 30 °C on a rotary shaker at 150 rpm for 10 days. At the end of incubation, 5 mL of culture was withdrawn, centrifuged at 5000 rpm for 10 minutes and removed cells and the cell-free supernatant was analyzed for residual chlorpyrifos. Chlorpyrifos degradation was measured Spectrophotometrically at 300 nm (λmax of chlorpyrifos) and the percentage degradation was calculated using the formula (Asamba *et al.,* 2022).

$$\text{Degradation (\%)} = \frac{A_{Control} - A_{Sample}}{A_{Control}}$$

where $A_{control}$ is the absorbance of uninoculated MSM with chlorpyrifos and A_{sample} is the absorbance of inoculated MSM after incubation. All experiments were performed in triplicate.

Time-course spectrophotometric degradation study

The most efficient isolates were selected for a time-course degradation assay. Each isolate was inoculated into 100 mL of MSM containing chlorpyrifos (50 µL L^{-1}) and incubated at 30 °C on a rotary shaker at 150 rpm for 10 days. Samples (5 mL) were withdrawn at 2-day intervals (days 0, 2, 4, 6, 8 and 10 days), centrifuged at 5000 rpm for 10 minutes and the supernatant was analyzed for residual chlorpyrifos at 300 nm. Percentage degradation was calculated. Uninoculated flasks served as controls (Asamba *et al.,* 2022).

Qualitative screening of extracellular enzyme production

Selected chlorpyrifos-degrading isolates were screened for extracellular hydrolytic enzyme production (amylase, cellulase and invertase) using plate assays (Dai *et al.,* 2020).

Amylase production

Isolates were spot-inoculated onto starch agar plates and incubated at 30 °C for 48 hours. After incubation, plates were flooded with 1% iodine solution. A clear zone around the colony indicated starch hydrolysis.

Cellulase production

Isolates were inoculated on to carboxymethyl cellulose (CMC) agar plates and incubated at 30 °C for 48–72 hours. Petri plates were flooded with 0.1% Congo red solution for 15 minutes followed by destaining with 1 M NaCl. A clear zone around growth indicated cellulase activity.

Invertase production

Isolates were inoculated on to sucrose agar plates and incubated at 30 °C for 48 hours. Plates were flooded with Benedict's reagent and heated at 80°C for 10 minutes. A colour changed from yellow to blue indicated the invertase activity.

All assays were performed in triplicate and enzyme production was scored as positive (+) or negative (–) based on zone formation or colour change.

Quantitative estimation of extracellular enzyme activity

The best-performing isolates were grown in 100 mL of respective substrate-containing broth (1% starch for amylase, 1% CMC for cellulase and 2% sucrose for invertase) at 30 °C on a rotary shaker at 150 rpm for 72 hours. Cultures were centrifuged at 10,000 rpm for 15 minutes at 4 °C and the cell-free supernatant was used as the crude enzyme source (Dai *et al.,* 2020).

Enzyme activity

Enzyme activity was expressed as U mL^{-1} of culture supernatant.

$$\text{Enzyme activity (U/mL)} = \frac{mg\ of\ reducing\ sugar\ released \times Dilution\ factor}{REaction\ time\ (min) \times Volume\ of\ enzyme\ (ml)}$$

Amylase activity

One milliliter of crude enzyme was mixed with 1 mL of 1% starch solution in 0.1 M phosphate buffer (pH 7.0) and incubated at 37 °C for 30 minutes. The reaction was terminated by added 2 mL of 3,5-dinitrosalicylic acid (DNS) reagent, followed by boiling for 10 minutes. After cooling, absorbance was measured at 540 nm. One unit (U) of amylase activity was defined as the amount of enzyme produced by 1 µmol of glucose per minute under assay condition.

Cellulase activity

One milliliter of crude enzyme was mixed with 1 mL of 1% CMC in 0.1 M phosphate buffer (pH 7.0) and incubated at 50 °C for 60 minutes. The reaction was stopped by adding 2 mL DNS reagent and boiling for 10 minutes. Absorbance was measured at 540 nm. One unit (U) of cellulase activity was defined as the amount of enzyme produced by 1 µmol of glucose per minute.

Invertase activity

One milliliter of crude enzyme was mixed with 1 mL of 2% sucrose solution in 0.1 M phosphate buffer (pH 5.0) and incubated at 37 °C for 30 minutes. The reaction was terminated with DNS reagent, boiled for 10 minutes and absorbance was read at 540 nm. One unit (U) of invertase activity was defined as the amount of enzyme produced by 1 µmol of reducing sugar (expressed as invert sugar) per minute.

Statistical analysis

All experiments were conducted in triplicate and data were expressed as mean ± standard deviation (SD).

RESULTS

The growth tolerance screening of the chlorpyrifos-degrading isolates showed that only a subset of bacteria could grow across increasing chlorpyrifos concentrations, indicating strain-specific resistance and utilization capacity. At 25 µL, 24 isolates showed visible growth, while 07 isolates were negative. At 50 µL, the number of growing isolates reduced further and only 15 isolates retained growth. At 75 µL, growth was observed in 13 isolates, whereas at 100 µL, only 6 isolates, namely *B. amyloliquefaciens, B. cereus, B. subtilis, Enterobacter* sp., *P.aeruginosa* and *P. mendocina* remained positive (Fig. 1, 2).

Among all isolates, *B. amyloliquefaciens, B. cereus, B. subtilis, Enterobacter* sp., *P. aeruginosa* and *P. mendocina* demonstrated the highest chlorpyrifos tolerance because they showed growth at all four concentrations tested. *B. albus*, *B. altitudinis*, *B. haynesii*, *B. tequilensis*, *M. hydrocarbonoclasticus*, *Planococcus* sp., *Pseudoalteromonas* sp., *S. arlettae* and *V. parahaemolyticus* were able to grow up to 75 µL but not at 100 µL. *Acinetobacter* sp., *A. aneurinilyticus*, *M. luteus*, *P. damselae*, *S. marcescens*, *Serratia* sp., *S. seohaensis*, *V. alginolyticus* and *V. harveyi* showed growth only at lower concentrations, suggesting moderate tolerance. Several isolates including *A. macleodii*, *B. licheniformis*, *Halomonas* sp., *S. bicestrii*, *S. putrefaciens*, *S. cohnii* and *S. saprophyticus* did not show growth at any concentration, indicated absence of chlorpyrifos tolerance.

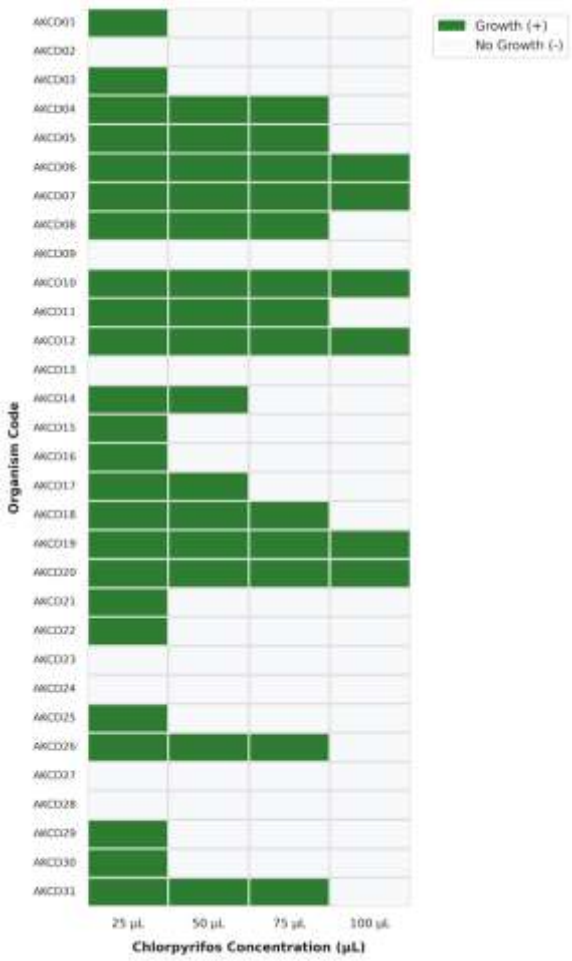


Fig. 1. Screening the growth tolerance of isolated bacteria by degrading chlorpyifos

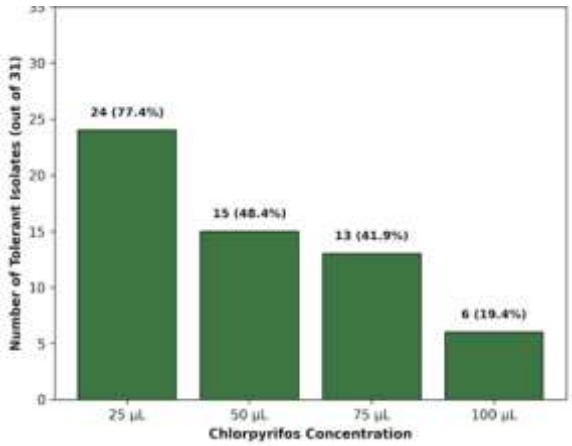


Fig. 2. Efficacy of bacterial growth response in different concentration

The most tolerant isolates identified by this screening were the same strains that showed strong degradation in the earlier quantitative assays, particularly *P. aeruginosa*, *P. mendocina*, *Enterobacter* sp., *B.*

cereus and *B. subtilis*. This suggested a positive relationship between growth tolerance and chlorpyrifos-degrading ability were performed.

Chlorpyrifos concentration growth

Out of 31 bacterial isolates screened for Chlorpyrifos growth tolerance, 24 strains (77.4%) exhibited tolerance at lower pesticide concentrations, while 6 strains (19.4%) demonstrated maximum growth tolerance up to 100 µL.

Isolate categorization by maximum tolerance level

Highest Tolerance (100 µL): Six isolates (*B. amyloliquefaciens, B. cereus, B. subtilis, Enterobacter sp., P. aeruginosa, P. mendocina*) represent priority candidates for investigations into high-concentration pesticide biodegradation (Fig. 3).

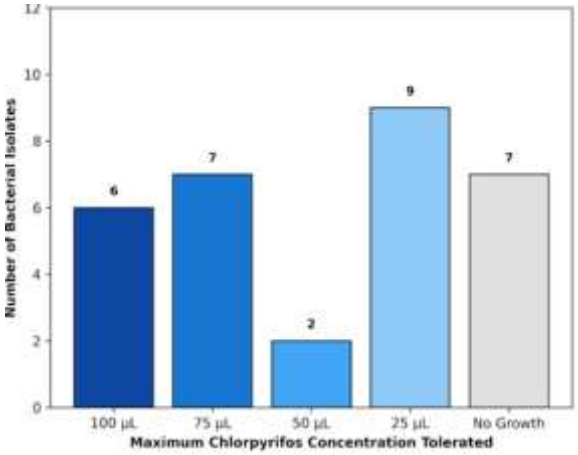


Fig. 3. Efficacy of maximum tolerance of chlorpyrifos in different concentration

High Tolerance (75 µL): Seven isolates (*B. albus, B. altitudinis, B. haynesii, B. tequilensis, Pseudoalteromonas* sp., *S. arlettae, V. parahaemolyticus*) demonstrate substantial tolerance capacity. Moderate Tolerance (50 µL): Two isolates (*M. hydrocarbonoclasticus, Planococcus* sp.) exhibit intermediate tolerance levels. Low Tolerance (25 µL): Nine isolates (*Acinetobacter* sp., *A. aneurinilyticus, M. luteus, P. damselae, S. marcescens, Serratia* sp., *S. seohaensis, V. alginolyticus, V. harveyi*) display minimal tolerance thresholds. Non-Tolerant (No Growth): Seven isolates (*A. macleodii, B. licheniformis, Halomonas* sp., *S. bicestrii, S. putrefaciens, S. cohnii, S. saprophyticus*) demonstrate growth inhibition even at the baseline 25 µL concentration.

The quantitative screening of chlorpyrifos-degrading bacteria showed that all isolates exhibited variable degradation potential in MSM, but the activity differed markedly among strains and substrate concentrations. Among the 31 isolates tested, *Enterobacter* sp., *P. aeruginosa*, *B. subtilis*, *B. amyloliquefaciens*, *P. mendocina* and *B. cereus* showed the highest degradation values in the CP + inoculum condition, particularly at 25 µL and 50 µL, indicating strong chlorpyrifos-utilizing ability.

At 25 µL chlorpyrifos concentration, *Enterobacter* sp recorded the maximum degradation of 57.36 ± 0.09%, followed closely by *P. aeruginosa* with 57.36 ± 0.04% and *B. subtilis* with 56.84 ± 0.32% (Fig. 4). *B. cereus* also showed high degradation of 50.52 ± 0.31%, while *B. amyloliquefaciens* and *P. mendocina* exhibited 46.31 ± 0.32% and 45.78 ± 0.14%, respectively. In contrast, several isolates such as *Acinetobacter* sp, *A.macleodii*, *A. aneurinilyticus*, *B. licheniformis*, *P. damselae* and *S. bicestrii* showed poor degradation, mostly below 6%, indicating weak or negligible chlorpyrifos degrading capacity.

At 50 µL, the strongest degradation was again observed in *P. aeruginosa* (45.07 ± 0.02%) and *Enterobacter* sp (42.25 ± 0.01%), followed by *B. cereus* (38.49 ± 0.21%) and *B. subtilis* (38.02 ± 0.12%). *P. mendocina* also maintained high activity with 37.67 ± 0.35%, while *B. amyloliquefaciens* showed 30.04 ± 0.12%. Lower values were noted in most other isolates, with *B. licheniformis* showing the least degradation at 1.40 ± 0.15%.

At 75 µL, *P. aeruginosa* remained the best performer with 39.42 ± 0.04%, followed by *P. mendocina* with 35.49 ± 0.13%, *Enterobacter* sp with 34.48 ± 0.32%, *B. cereus* with 32.75 ± 0.34% and *B. subtilis* with 30.70 ± 0.09%. At 100 µL, the highest degradation was observed in *P. aeruginosa* (38.51 ± 0.04%) and *P.*

mendocina (34.58 ± 0.42%), followed by *Enterobacter* sp (31.49 ± 0.04%), *B. cereus* (30.70 ± 0.28%) and *B. subtilis* (27.55 ± 0.03%). Overall, degradation tended to decline as chlorpyrifos concentration increased, but the efficient isolates maintained appreciable activity even at the highest dose.

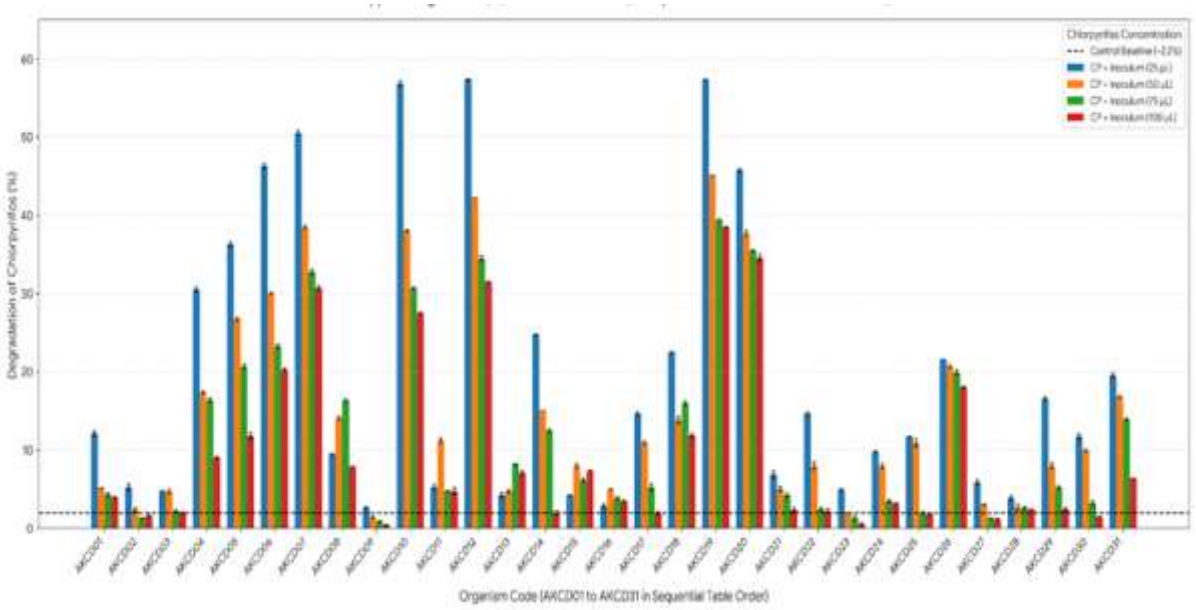


Fig. 4. Efficacy of degradation of chlorpyrifos by different bacterial strains

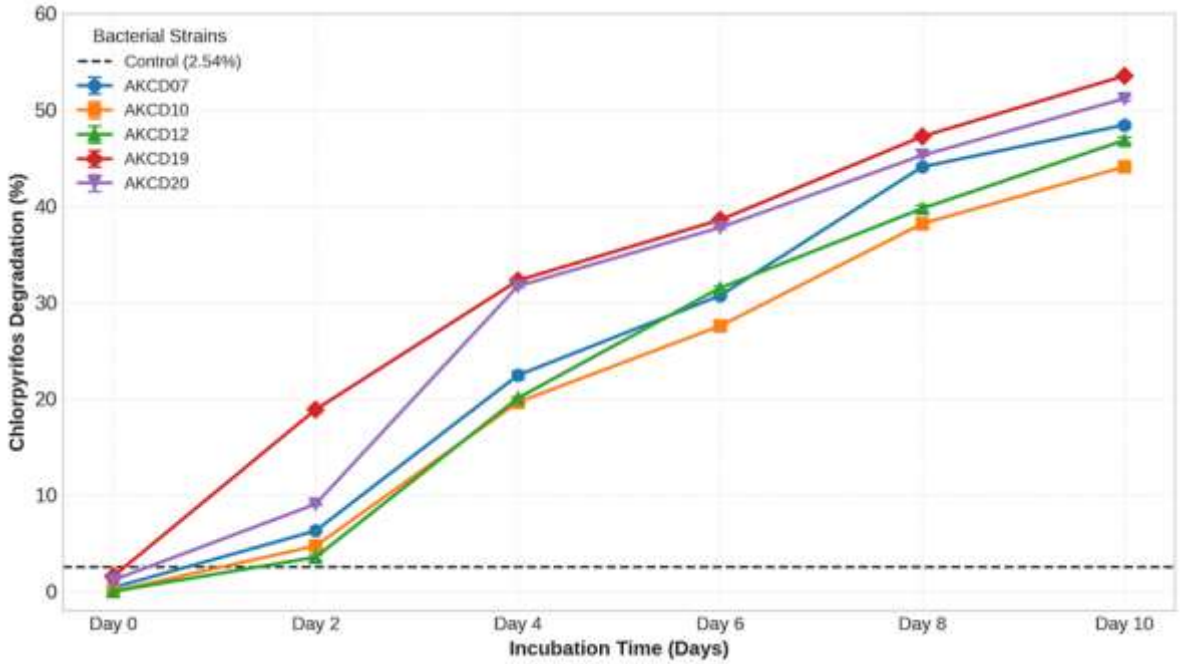


Fig. 5. Spectrophotometric degradation assessment of chlorpyrifos

In the Spectrophotometric degradation assay over 10 days, the selected isolates also showed progressive chlorpyrifos degradation with incubation time. *P. aeruginosa* exhibited the highest degradation, increasing from 1.57 ± 0.09% on day 0 to 53.54 ± 0.03% on day 10. *P. mendocina* reached 51.18 ± 0.23% on day 10, while *B. cereus*, *B. subtilis* and *Enterobacter* sp., recorded 48.43 ± 0.21%, 44.09 ± 0.11% and 46.85 ± 0.32%, respectively (Fig. 5). This time-dependent increase confirmed that these isolates actively degraded chlorpyrifos during incubation.

Table 2. Primary screening of extracellular enzyme by the potential chlorpyrifos-degrading bacteria

Name of the bacteria	Presence of enzyme		
	Amylase	Cellulose	Invertase
B. subtilis	++	+	+
B. cereus	+	-	+
P.aeruginosa	++	+	++
P.mendocina	+	+	-
Enterobacter sp.	+	-	-

(++) Strongly Present, (+) Present, (-) Absent

The primary qualitative screening of extracellular enzyme production by the chlorpyrifos-degrading bacterial isolates showed that all five strains expressed one or more hydrolytic enzymes, though the pattern varied among organisms. *B. subtilis* exhibited strong amylase activity and positive cellulase and invertase production. *B. cereus* showed amylase and invertase activity but lacked cellulase. *P. aeruginosa* displayed the broadest enzyme profile, with strong production of amylase, cellulase and invertase. *P. mendocina* was positive for amylase and cellulase but negative for invertase, while *Enterobacter* sp. showed only amylase production.

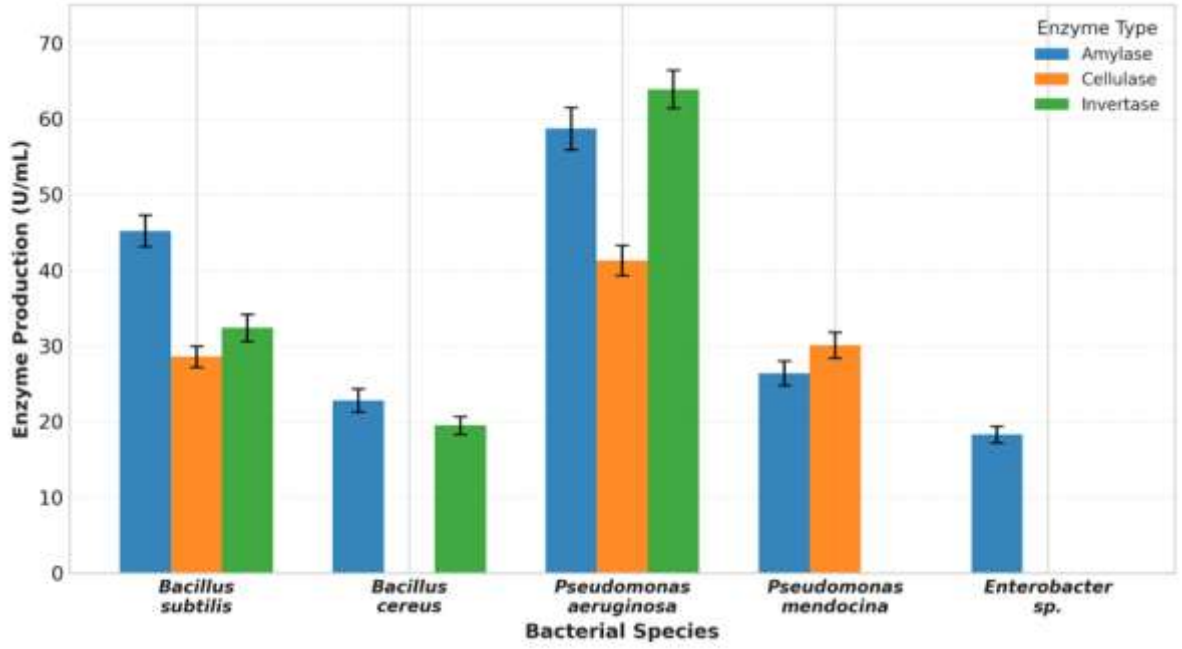


Fig. 6. Estimating production of extracellular enzyme by the potential chlorpyrifos-degrading bacteria

The quantitative estimation confirmed this trend. *P. aeruginosa* recorded the highest enzyme activities, with amylase production of 58.7 ± 2.8 U/mL, cellulase production of 41.3 ± 2.0 U/mL and invertase production of 63.9 ± 2.5 U/mL. *B. subtilis* followed with 45.2 ± 2.1 U/mL amylase, 28.6 ± 1.4 U/mL cellulase and 32.4 ± 1.8 U/mL invertase. *P. mendocina* produced 26.4 ± 1.6 U/mL amylase and 30.1 ± 1.7 U/mL cellulase, but invertase was not detected. *B. cereus* showed 22.8 ± 1.5 U/mL amylase and 19.5 ± 1.2 U/mL invertase, whereas *Enterobacter* sp. exhibited the lowest enzyme production with 18.3 ± 1.1 U/mL amylase and no detectable cellulase or invertase (Fig. 6).

DISCUSSION

The growth tolerance assay clearly indicated that chlorpyrifos imposes selective pressure on the bacterial isolates, permitting only resistant strains to survive and grow at higher concentrations. The ability of *B. amyloliquefaciens*, *B. cereus*, *B. subtilis*, *Enterobacter* sp., *P. aeruginosa* and *P. mendocina* to grow even at 100 µL suggested that these isolates possess physiological and metabolic adaptations enabling them to withstand pesticide stress. Such tolerance is often associated with chlorpyrifos-degrading bacteria that can use the compound as a carbon, phosphorus, or energy source (Lagiso *et al.*, 2026).

Elevated pesticide level previously reported in lake, likely stemming from intensive agricultural applications (Deribe *et al.*, 2011) underscores the urgency of ecofriendly CP removal strategies, given its toxicity to humans, animals and microbes (Aswathi *et al.,* 2019; Pathak *et al.,* 2022).

The reduction in the number of positive isolates with increasing chlorpyrifos concentration is expected because higher pesticide levels can inhibit membrane function, enzyme activity and overall cellular metabolism. Similar concentration-dependent growth inhibition has been reported in earlier chlorpyrifos tolerance studies, where only a few bacterial strains were able to grown at high pesticide doses while many others were suppressed (Dwivedi *et al.*, 2021).

The strong performance of *P. aeruginosa* and *P. mendocina* is particularly important because these isolates also showed the highest degradation percentages in the quantitative assay and the highest final degradation in the time-course Spectrophotometric study. This consistency supports the view that growth tolerance is closely linked with biodegradation potential.

Bacteria that tolerate chlorpyrifos well are more likely to survive long enough to express degradative enzymes and complete pesticide breakdown. In a prior study, (Saengsanga and Phakratok, 2023), (Asamba *et al.,* 2022) and (Omeiri *et al.,* 2024) observed increased degradation of chlorpyrifos (>90%). Another study however, reported relatively lower degradation (52%) and (57.8%) of chlorpyrifos within 14 and 21 days of incubation (Ewida, 2023; Saengsanga and Phakratok, 2023).

From a bioremediation perspective, the tolerant isolates are the most promising candidates for further work because they can remain active under pesticide stress. Their survival at higher chlorpyrifos levels suggests potential application in contaminated soils or water systems, where pesticide concentration may initially be inhibitory to less adapted microbes. The data therefore support the selection of *P. aeruginosa*, *P. mendocina*, *Enterobacter* sp., *B. cereus* and *B. subtilis* for molecular identification, enzyme profiling and future bioremediation studies. The previous study isolated microbial strains that degrade chlorpyrifos, with most strains isolated being rod-shaped and belonging to both Gram groups (Asamba *et al.*, 2022).

The present findings demonstrate that several bacterial isolates possess significant potential to degrade chlorpyrifos, but the efficiency varies among strains and is influenced by substrate concentration and incubation period. The strongest performers, especially *P. aeruginosa*, *Enterobacter* sp., *B. subtilis*, *P. mendocina* and *B. cereus*, likely possess chlorpyrifos-hydrolyzing enzymes or metabolic pathways that enable them to use the pesticide or its intermediates as carbon and/or energy sources. Similar observations have been reported for chlorpyrifos-degrading bacteria such as *Pseudomonas*, *Enterobacter*, *Bacillus* and *Achromobacter*, which are known to show efficient degradation in mineral salt media (Ghorab *et al.*, 2026).

The decreasing trend in degradation with increasing chlorpyrifos concentration suggests a possible inhibitory effect of higher pesticide loads on bacterial growth and enzyme activity.

At lower concentrations, the bacteria may more easily adapt and initiate hydrolysis of chlorpyrifos, whereas higher concentrations can impose toxicity and reduce metabolic efficiency (Noctor *et al.,* 2024). This pattern is consistent with earlier reports where chlorpyrifos degradation was highest at lower or moderate pesticide concentrations and diminished when the compound level increased beyond the tolerance range of the strain.

The progressive increase in degradation over 10 days in the Spectrophotometric assay indicates that degradation was not immediate but occurred in a time-dependent manner. This suggests that the

organisms required an adaptation phase before actively metabolizing chlorpyrifos, after which degradation increased steadily. Previous studies have shown that bacterial degradation of chlorpyrifos in MSM commonly improves with incubation time, with several strains reaching substantial degradation after 7–16 days depending on inoculum density, conditions and strain capability (Akbar and Sultan, 2016; Olanrewaju *et al.,* 2017).

Among the isolates, *P. aeruginosa* was the most efficient degrader in both concentration-based and time-based assays, indicating strong biodegradation potential and making it a promising candidate for further characterization. *P. mendocina*, *Enterobacter* sp., *B. cereus* and *B. subtilis* also showed consistent performance, suggesting they may be suitable for development as bioremediation agents. The comparatively poor performance of many isolates indicates that chlorpyrifos degradation is strain-specific and depends on the presence of suitable catabolic enzymes, adaptation to pesticide stress and overall physiological fitness.

The extracellular enzyme profile indicated that the chlorpyrifos-degrading isolates are metabolically versatile and capable of secreting hydrolytic enzymes that may support their survival and activity in pesticide-contaminated environments. The strong enzyme production observed in *P. aeruginosa* (Gilani *et al.,* 2016) and *B. subtilis* suggests that these isolates are physiologically robust and better adapted to exploit complex organic substrates. Such enzymatic versatility is often advantageous in contaminated sites, where microbes must tolerate stress while maintaining nutrient acquisition and growth (Chishti *et al.,* 2013).

Among the isolates, *P. aeruginosa* was the most potent enzyme producer, which may be linked to its superior degradation and growth tolerance performance in earlier screening assays. *Pseudomonas* species are widely recognized for their broad metabolic capacity and environmental adaptability and they are frequently reported among pesticide-degrading bacteria. The high amylase, cellulase and invertase activities of *P. aeruginosa* suggest strong extracellular secretion potential, which can aid colonization and persistence in chlorpyrifos-amended media.

B. subtilis also showed a strong enzyme pattern, particularly for amylase and invertase. This is consistent with the known enzymatic competence of *Bacillus* species, which are commonly associated with biodegradation and bioremediation applications due to their ability to secrete large amounts of extracellular enzymes and withstand harsh environmental conditions. The presence of cellulase further indicates that *B. subtilis* can utilize diverse carbon sources, enhancing its survival under pesticide stress.

The comparatively lower enzyme production in *B. cereus* and *Enterobacter* sp. suggests species-specific differences in enzyme expression and metabolic potential. The absence of cellulase in *B. cereus* and the absence of both cellulase and invertase in *Enterobacter* sp. indicate a narrower enzymatic repertoire, which may limit their ecological fitness relative to *P. aeruginosa* and *B. subtilis* (Gilani *et al.,* 2016). Similar variability in hydrolytic enzyme production has been reported in pesticide-degrading bacteria, where some strains show broad extracellular activity while others express only one or two enzymes (Ambreen and Yasmin, 2020).

Overall, the data suggested that *P. aeruginosa* and *B. subtilis* are the most promising isolates for further study because they combine strong chlorpyrifos tolerance, high degradation ability and substantial extracellular enzyme production. These features make them suitable candidates for molecular identification, enzymatic characterization and future bioremediation applications. The data clearly indicated that the selected isolates, particularly *P. aeruginosa*, *P. mendocina*, *Enterobacter* sp., *B. cereus* and *B. subtilis*, can effectively degrade chlorpyrifos under laboratory conditions. These isolates may therefore be considered for further

molecular identification, enzyme analysis and field-scale bioremediation studies.

CONCLUSION

The present study suggested that chlorpyrifos-degrading potential among the bacterial isolates was highly strain-specific. Although all isolates were exposed to the same conditions, only a few strains showed strong tolerance, sustained growth and appreciable degradation activity, with *P. aeruginosa*, *P. mendocina*, *Enterobacter* sp., *B. cereus* and *B. subtilis* emerging as the most efficient isolates. The decrease in degradation at higher chlorpyrifos concentrations, along with continued activity by the best-performing strains, confirms their adaptability and bioremediation potential. The enzyme analysis further supported the degradation results, as the superior isolates were also able to produce hydrolytic enzymes such as amylase, cellulase and invertase. In particular, *P. aeruginosa* showed the highest enzyme production and the greatest chlorpyrifos degradation, suggesting that its metabolic versatility may contribute to pesticide breakdown. Therefore, these isolates, especially *P. aeruginosa,* can be considered promising candidates for further optimization and application in the bioremediation of chlorpyrifos-contaminated environments.

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