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Isolation and identification of marine bacterial strains on the efficiency of degrading chlorpyrifos

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ABSTRACT

Chlorpyrifos is a widely used organophosphate pesticide that poses severe environmental and health risks due to its persistence in ecosystems. This study aimed to isolate marine bacteria from marine waters of Kerala (Cochin, Kollam and Calicut) and screen their potential for chlorpyrifos degradation. A total of 30 bacterial strains were isolated and characterised using morphological (colony shape, surface, margin, color, elevation, consistency, opacity) and biochemical tests (Gram staining, indole production, methyl red, Voges-Proskauer, citrate utilization, catalase, carbohydrate fermentation, triple sugar iron, urease, coagulase, DNase, oxidase and lecithinase tests). The isolates belonged to diverse genera including *Acinetobacter, Bacillus, Pseudomonas, Vibrio, Serratia, Shewanella, Alteromonas, Pseudoalteromonas, Photobacterium, Staphylococcus, Enterobacter, Halomonas and Marinobacter*. Growth tolerance screening for chlorpyrifos degradation (25–100 µL) revealed five isolates (AKCD06, AKCD07, AKCD10, AKCD19 and AKCD20) capable of surviving at the highest concentration (100 µL). Among these, AKCD07, AKCD10, AKCD12, AKCD19 and AKCD20 were identified as the most promising degraders. Notably, AKCA07 (*Pseudomonas aeruginosa*) from Calicut demonstrated superior chlorpyrifos degradation potential compared to all other strains, attributed to its organophosphate hydrolase (OPH) enzyme activity. These marine bacterial isolates, particularly *P. aeruginosa* AKCA07, represent valuable candidates for bioremediation of chlorpyrifos-contaminated environments and warrant further investigation through quantitative degradation assays, enzyme characterization and field trials.

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INTRODUCTION

Chlorpyrifos (O,O-diethyl-O-3,5,6-trichloro-2-pyridyl phosphorothioate) ranks among the most extensively utilized broad-spectrum organophosphate insecticides worldwide, finding significant application in agriculture, forestry and residential areas for the management of insect pests affecting crops such as vegetables, fruits, cotton and rice (Wang *et al.*, 2010). Despite its efficacy in pest control, the persistence of chlorpyrifos in the environment presents considerable risks due to its high toxicity, potential for bioaccumulation and neurotoxic impacts on non-target species, including fish, aquatic invertebrates and humans. This pesticide functions by inhibiting acetyl cholinesterase, which results in neurological impairment (Verma *et al.*, 2016).

Environmental contamination arises from agricultural runoff, leaching and atmospheric deposition (Ghiglione *et al.,* 2016). Agricultural runoff often carries chlorpyrifos residues into water reservoirs, leading to the contamination of water source, which poses a threat to biodiversity (Jacquin *et al.,* 2019). Residues of chlorpyrifos have been identified in sediments, water and organisms, with concentrations surpassing permissible limits in various areas (Gu and Wang, 2014). The half-life of chlorpyrifos in aquatic environments ranges from 4 to 120 days, influenced by factors such as pH, temperature, sunlight and microbial activity.

Traditional physicochemical remediation techniques tend to be costly and may generate toxic by-products. Conversely, bioremediation utilizing microorganisms presents an environmentally friendly and economically viable alternative. Certain bacteria have demonstrated remarkable capabilities for the degradation of chlorpyrifos, attributed to their rapid growth rates, metabolic flexibility and capacity to thrive in a variety of environmental conditions.

Multiple bacterial genera have been identified as potential degraders of chlorpyrifos, including *Pseudomonas, Bacillus, Enterobacter, Stenotrophomonas, Virgibacillus, Vibrio, Arthrobacter, Flavobacterium, Citrobacter* and *Rhizobium*. Notably, species of *Virgibacillus* isolated from marine bivalves have exhibited remarkable degradative capabilities, utilizing chlorpyrifos as their sole carbon and energy source for growth in saline environments (Singh *et al.,* 2026).

Research has highlighted significant degradation efficiencies among marine bacterial isolates. The *Virgibacillus salarius* strain accomplished a degradation rate of 68.14% within 4 days at a concentration of 50 mg L^{-1} chlorpyrifos, while the *Pseudomonas aeruginosa* strain achieved an 85.5% degradation rate within 7 days. Furthermore, bacterial consortia have demonstrated even greater efficiency, reaching degradation rates of 80–97% within 48–96 hours under optimized conditions. The degradation pathway generally involves the hydrolysis of the P-O bond by organophosphate hydrolase (OPH), resulting in the formation of 3,5,6-trichloro-2-pyridinol (TCP) and diethyl phosphorothioate as primary metabolites, followed by the subsequent degradation of TCP.

Nevertheless, the degradation of marine bacterial isolates to varying concentrations of chlorpyrifos remains inadequately explored, especially for strains originating from tropical marine environments, such as those found along the Indian marine. Previous research has predominantly concentrated on soil-dwelling or freshwater bacteria, with insufficient focus on marine isolates that are adapted to saline conditions.

It is crucial to screen isolates for growth tolerance across a range of chlorpyrifos concentrations (100–2000 ppm) to identify resilient degraders that are suitable for large-scale bioremediation efforts. Gaining an understanding of growth responses under pesticide stress is vital for elucidating bacteria in the remediation of degrading contaminated ecosystems.

MATERIALS AND METHODS

Sample collection

Marine water samples were collected from Kochi, Kollam and Calicut of kerala aseptically in sterile,

screw-capped containers from the specified sampling site. Each container was meticulously labeled with the date and time of collection. The samples were subsequently transported to the laboratory in an ice box and processed immediately after collection.

Isolation of bacteria

The water samples were subjected to serial dilution (10^{-4}, 10-*5* and 10^{-6}) using either sterile saline or sterile seawater. Following this, 0.1 mL of each dilution was spread onto 3% NaCl nutrient agar plates using a sterile glass spreader. The plates were incubated at temperatures between 28 and 37°C for a period of 24 to 72 hours. After incubation, various bacterial colonies were assessed based on their morphology, color, size, elevation and margin. Distinct colonies were chosen and streaked multiple times on fresh 3% NaCl nutrient agar plates to obtain pure cultures. The purified isolates were then preserved on agar slants or in glycerol stocks.

Identification of bacteria

The pure isolates underwent Gram staining to determine cell shape, arrangement and Gram reaction. Colony characteristics (Sheikh *et al.,* 2024) were recorded based on pigmentation, texture, form and surface appearance. Further characterization of the isolates was conducted using standard biochemical tests (Shoaib *et al.,* 2020), including catalase, oxidase, motility, indole, methyl red, Voges-Proskauer, citrate utilization, urease and sugar fermentation tests.

The results were interpreted according to established bacteriological methods. The bacterial isolates were identified based on their colony morphology, microscopic characteristics and biochemical profile results. The confirmed isolates were stored appropriately for future reference and analysis.

Screening the growth tolerance of isolated bacteria by degrading chlorpyifos

Bacterial isolates previously obtained from marine water samples of Kerala (Cochin, Kollam and Calicut) were screened for chlorpyrifos degradation ability using Mineral Salts Medium (MSM) as the basal medium. Bacteria were stored in Nutrient broth to obtain cell density of approximately 10^8 CFU/mL at 0.5 McFarland standard. Chlorpyrifos stock solution was prepared and added to sterilized MSM medium at various concentrations to serve as the sole carbon source. Bacterial inoculum was prepared by activating isolates on MSM agar plates, followed by chlorpyrifos in different concentration (25, 50, 75and100 µL) Each bacterial isolate was inoculated into chlorpyrifos supplemented MSM broth in different concentration (25, 50, 75 and 100 µL) and incubated at 28±2°C on a rotary shaker at 120 rpm for 7 days.

Degradation was monitored visually and spectrophotometrically at 600 nm every 24 hours, with growth recorded as present or absent based on visible turbidity after the incubation period. controls (MSM broth without chlorpyrifos) were included to ensure experimental validity. Experiments were performed in triplicate and isolates demonstrating growth at the highest chlorpyrifos concentration were selected as potential degraders for further characterization. All experiments were conducted following appropriate biosafety protocols for handling hazardous organophosphate pesticides in a laboratory.

RESULTS

Isolation and morphological characterization of marine bacterial strains

A total of 30 bacterial strains were isolated from marine water samples collected from three coastal locations in Kerala: Cochin (14 strains: AKCO01–AKCO14), Kollam (15 strains: AKKO01–AKKO15) and Calicut (15 strains: AKCA01–AKCA15) (Table 1). Morphological characterization revealed diverse colony characteristics among the isolates. Growth rates varied from slow to rapid, with most isolates exhibiting moderate growth. Colony shapes were predominantly circular or irregular. Colony surfaces were either smooth (majority) or rough. Margins included entire (smooth edge), undulate (wavy) and lobate (marked indentations). Colony

colors ranged from off-white, cream, creamy white, yellow, red (prodigiosin pigment in AKKO12 and AKCA08, indicative of *Serratia marcescens*), grayish, greenish (AKKO11 and AKCA07, characteristic of *Pseudomonas* pigment production), to red/cream. Elevations were flat, raised, or convex. Consistencies included mucoid, moist and dry and all isolates were opaque.

Table 1. Morphological characterization of isolated bacteria from the marine water sample of Cochin, Kollam and Calicut

Bacterial code	Bacteria	Cochin							
		Growth	Shape	Surface	Margin	Color	Elevation	Consistency	Opacity
AKCO01	*Acinetobacter* sp	Moderate	Circular	Smooth	Entire	Off-white	Raised	Mucoid	Opaque
AKCO02	*Alteromonas macleodii*	Moderate	Irregular	Smooth	Undulate	Cream	Raised	Moist	Opaque
AKCO03	*Bacillus altitudinis*	Moderate	Circular	Rough	Entire	Cream	Raised	Dry	Opaque
AKCO04	*Bacillus amyloliquefaciens*	Rapid	Irregular	Rough	Undulate	Creamy white	Flat	Dry	Opaque
AKCO05	*Bacillus cereus*	Rapid	Irregular	Rough	Lobate	Off-white	Flat	Dry	Opaque
AKCO06	*Bacillus licheniformis*	Moderate	Irregular	Rough	Lobate	Cream	Raised	Dry	Opaque
AKCO07	*Bacillus subtilis*	Rapid	Irregular	Rough	Lobate	Off-white	Flat	Dry	Opaque
AKCO08	*Marinobacter hydrocarbonoclasticus*	Moderate	Circular	Smooth	Entire	Cream	Convex	Mucoid	Opaque
AKCO09	*Planococcus* sp	Moderate	Circular	Smooth	Entire	Yellow	Convex	Dry	Opaque
AKCO10	*Pseudoalteromonas* sp	Moderate	Circular	Smooth	Entire	Cream/Yellow	Convex	Moist	Opaque
AKCO11	*Pseudomonas mendocina*	Moderate	Circular	Smooth	Entire	Cream/Yellow	Raised	Moist	Opaque
AKCO12	*Serratia* sp	Moderate	Circular	Smooth	Entire	Red/Cream	Convex	Moist	Opaque
AKCO13	*Shewanella bicestrii*	Moderate	Irregular	Smooth	Undulate	Cream	Raised	Moist	Opaque
AKCO14	*Vibrio alginolyticus*	Rapid	Circular	Smooth	Entire	Cream	Convex	Moist	Opaque
Bacterial code	Bacteria	Kollam							
		Growth	Shape	Surface	Margin	Color	Elevation	Consistency	Opacity
AKKO01	*Acinetobacter* sp	Moderate	Circular	Smooth	Entire	Off-white	Raised	Mucoid	Opaque
AKKO02	*Bacillus albus*	Moderate	Irregular	Rough	Lobate	White	Flat	Dry	Opaque
AKKO03	*Bacillus cereus*	Rapid	Irregular	Rough	Lobate	Off-white	Flat	Dry	Opaque
AKKO04	*Bacillus licheniformis*	Moderate	Irregular	Rough	Lobate	Cream	Raised	Dry	Opaque
AKKO05	*Bacillus subtilis*	Rapid	Irregular	Rough	Lobate	Off-white	Flat	Dry	Opaque
AKKO06	*Bacillus tequilensis*	Moderate	Circular	Smooth	Entire	Cream	Raised	Dry	Opaque
AKKO07	*Enterobacter* sp	Moderate	Circular	Smooth	Entire	Creamy white	Convex	Mucoid	Opaque
AKKO08	*Halomonas* sp	Moderate	Circular	Smooth	Entire	Cream/Yellow	Raised	Moist	Opaque
AKKO09	*Micrococcus luteus*	Slow	Circular	Smooth	Entire	Yellow	Convex	Dry	Opaque
AKKO10	*Photobacterium damselae*	Moderate	Circular	Smooth	Entire	Grayish	Convex	Moist	Opaque
AKKO11	*Pseudomonas aeruginosa*	Rapid	Irregular	Smooth	Undulate	Greenish	Flat	Mucoid	Opaque
AKKO12	*Serratia marcescens*	Moderate	Circular	Smooth	Entire	Red	Convex	Moist	Opaque
AKKO13	*Staphylococcus cohnii*	Moderate	Circular	Smooth	Entire	Cream	Convex	Dry	Opaque
AKKO14	*Vibrio alginolyticus*	Rapid	Circular	Smooth	Entire	Cream	Convex	Moist	Opaque
AKKO15	*Vibrio harveyi*	Rapid	Circular	Smooth	Entire	Yellow	Convex	Moist	Opaque
Bacterial code	Bacteria	Calicut							
		Growth	Shape	Surface	Margin	Color	Elevation	Consistency	Opacity
AKCA01	*Acinetobacter* sp	Moderate	Circular	Smooth	Entire	Off-white	Raised	Mucoid	Opaque
AKCA02	*Aneurinibacillus aneurinilyticus*	Moderate	Circular	Smooth	Entire	Yellowish	Flat	Dry	Opaque
AKCA03	*Bacillus haynesii*	Moderate	Circular	Smooth	Entire	White	Raised	Dry	Opaque
AKCA04	*Bacillus subtilis*	Rapid	Irregular	Rough	Lobate	Off-white	Flat	Dry	Opaque
AKCA05	*Photobacterium damselae*	Moderate	Circular	Smooth	Entire	Grayish	Convex	Moist	Opaque
AKCA06	*Planococcus* sp	Moderate	Circular	Smooth	Entire	Yellow	Convex	Dry	Opaque
AKCA07	*Pseudomonas aeruginosa*	Rapid	Irregular	Smooth	Undulate	Greenish	Flat	Mucoid	Opaque
AKCA08	*Serratia marcescens*	Moderate	Circular	Smooth	Entire	Red (prodigiosin)	Convex	Moist	Opaque
AKCA09	*Shewanella putrefaciens*	Moderate	Irregular	Smooth	Undulate	Cream	Raised	Moist	Opaque
AKCA10	*Shewanella seohaensis*	Moderate	Irregular	Smooth	Undulate	Grayish	Raised	Moist	Opaque
AKCA11	*Staphylococcus arlettae*	Moderate	Irregular	Smooth	Undulate	Cream	Raised	Moist	Opaque
AKCA12	*Staphylococcus cohnii*	Moderate	Circular	Smooth	Entire	Cream	Convex	Dry	Opaque
AKCA13	*Staphylococcus saprophyticus*	Moderate	Circular	Smooth	Entire	Cream	Convex	Dry	Opaque
AKCA14	*Vibrio harveyi*	Rapid	Circular	Smooth	Entire	Yellow	Convex	Moist	Opaque
AKCA15	*Vibrio parahaemolyticus*	Rapid	Circular	Smooth	Entire	Grayish	Convex	Moist	Opaque

Biochemical characterization of isolated strains

Biochemical characterization revealed that Gram-positive bacteria (AKCO03, AKCO04, AKCO05, AKCO06, AKCO07, AKKO02, AKKO03, AKKO04, AKKO05, AKKO06, AKKO09, AKKO13, AKCA02, AKCA03, AKCA04, AKCA05, AKCA06, AKCA12, AKCA13) were predominantly from the genus *Bacillus*. Gram-negative bacteria included *Acinetobacter*, *Alteromonas*, *Marinobacter*, *Planococcus*, *Pseudoalteromonas*, *Pseudomonas*, *Serratia*, *Shewanella*, *Staphylococcus*, *Enterobacter*, *Halomonas*, *Micrococcus*, *Photobacterium*, *Vibrio*, *Aneurinibacillus* and *Bacillus* species. Catalase activity was positive in all isolates, indicating aerobic or facultative anaerobic metabolism (Table 2). Citrate utilization was positive across all strains, demonstrating versatile carbon source utilization. Carbohydrate fermentation was positive in most isolates. Triple sugar iron (TSI) agar reactions showed alkaline/alkaline (K/K)

for *Acinetobacter* spp. (AKCO01, AKKO01, AKCA01), acidic/acidic (A/A) for many Gram-negative enterics and alkaline/acidic (K/A) for AKCO05, AKKO04 and some species. The oxidase test was positive for marine Gram-negative isolates including AKCO02, AKCO10, AKCO11–AKCO14, AKKO11, AKCA07, AKKO12, AKCA08, AKCA09–AKCA11, AKCA14 and AKCA15, confirming their marine origin. Indole production was positive for AKCO10, AKCO12, AKKO10, AKKO12, AKCA08, AKCA09, AKCA14 and AKCA15. Urease activity was positive for AKCO05 and AKCO13. DNase activity was positive for AKCO02–AKCO08, AKKO02–AKKO06, AKKO12, AKCA02–AKCA04, AKCA08 and AKCA09. Lecithinase activity was positive for AKCO05, indicating phospholipase production associated with pathogenicity.

Table 2. Biochemical characterization of isolated bacteria from the marine water sample of Cochin

Cochin													
Code	Gram staining	Indole production	Methyl red test	Voges proskaur test	Citrate utilization test	Catalase test	Carbohydrate fermentation test	Triple sugar	Urease test	Coagulase test	DNase test	Oxidase test	Lecithinase test
AKCO01	-	-	-	-	+	+	+	K/K	-	-	-	-	-
AKCO02	-	-	-	-	+	+	+	K/K	-	-	-	+	-
AKCO03	+	-	+	-	+	+	+	A/A	-	-	+	-	-
AKCO04	+	-	+	+	+	+	+	A/A	-	-	+	-	-
AKCO05	+	-	+	+	+	+	+	K/A	+	+	+	-	+
AKCO06	+	-	+	+	+	+	+	K/A	-	-	+	-	-
AKCO07	+	-	-	+	+	+	+	A/A	-	-	+	-	-
AKCO08	-	-	-	-	+	+	-	K/K	-	-	+	+	-
AKCO09	+	-	-	+	+	+	+	A/A	-	-	-	-	-
AKCO10	-	+	-	-	+	+	+	A/A	-	-	-	+	-
AKCO11	-	-	-	-	+	+	+	A/A	-	-	-	+	-
AKCO12	-	+	-	+	+	+	+	A/A	-	-	+	-	-
AKCO13	-	-	-	-	+	+	+	A/A	+	-	-	+	+
AKCO14	-	+	-	-	+	+	+	A/A	-	-	-	+	-

Kollam													
Code	Gram staining	Indole production	Methyl red test	VP test	Citrate Utilization test	Catalase test	Carbohydrate Fermentation test	Triple sugar	Urease test	Coagulase test	DNase test	Oxidase test	Lecithinase test
AKKO01	-	-	-	-	+	+	+	K/K	-	-	-	-	-
AKKO02	+	-	+	+	+	+	+	A/A	-	-	+	-	-
AKKO03	+	-	+	-	+	+	+	A/A	-	-	+	-	-
AKKO04	+	-	+	+	+	+	+	K/A	-	-	+	-	-
AKKO05	+	-	-	+	+	+	+	A/A	-	-	+	-	-
AKKO06	+	-	-	+	+	+	+	K/A	-	-	+	-	-
AKKO07	-	-	-	+	+	+	+	A/A	+	-	-	-	-
AKKO08	-	-	-	-	+	+	+	K/A	-	-	-	+	-
AKKO09	+	-	-	-	+	+	-	K/NC	-	-	-	-	-
AKKO10	-	+	-	-	+	+	+	A/A	-	-	-	+	+
AKKO11	-	-	-	-	+	+	-	A/A	-	-	-	+	-
AKKO12	-	+	-	+	+	+	+	A/A	-	-	+	-	-
AKKO13	+	-	-	+	-	+	+	A/A	-	-	-	-	-
AKKO14	-	+	-	-	+	+	+	A/A	-	-	-	+	-
AKKO15	-	+	-	-	+	+	+	K/A	-	-	-	+	-

Calicut													
Code	Gram staining	Indole production	Methyl red test	Voges proskaur test	Citrate utilization test	Catalase test	Carbohydrate fermentation test	Triple sugar	Urease test	Coagulase test	DNase test	Oxidase test	Lecithinase test
AKCA01	-	-	-	-	+	+	+	K/K	-	-	-	-	-
AKCA02	+	-	+	-	+	+	+	A/A	-	-	+	-	-
AKCA03	+	-	+	+	+	+	+	A/A	-	-	+	-	-
AKCA04	+	-	-	+	+	+	+	A/A	-	-	+	-	-
AKCA05	+	-	-	+	+	+	+	A/A	-	-	-	-	-
AKCA06	+	-	-	+	+	+	+	A/A	-	-	-	-	-
AKCA07	-	-	-	-	+	+	-	A/A	-	-	-	+	-
AKCA08	-	+	-	+	+	+	+	A/A	-	-	+	-	-
AKCA09	-	+	-	+	+	+	+	A/A	-	-	+	-	-
AKCA10	-	-	-	-	+	+	+	A/A	+	-	-	+	+
AKCA11	-	-	-	-	+	+	+	A/A	-	-	-	+	+
AKCA12	+	-	-	+	-	+	+	A/A	-	-	-	-	-
AKCA13	+	-	-	+	-	+	+	A/A	-	+	-	-	-
AKCA14	-	+	-	-	+	+	+	K/A	-	-	-	+	-
AKCA15	-	+	-	-	+	+	+	K/A	-	-	-	+	-

Alkaline slant/Alkaline butt (K/K), Alkaline slant/Acidic butt (K/A), Acidic slant/Acidic butt (A/A), Alkaline slant/no change in butt (K/NC), Positive (+)Negative (-)

Identification of key chlorpyrifos degradation candidate

The identification of AKCA07 (*Pseudomonas aeruginosa*) from marine water is particularly significant. This Gram-negative, oxidase-positive, catalase-positive, rapid-growing, greenish-pigmented, mucoid, irregular colony with undulate margin and flat elevation is well-documented for its exceptional chlorpyrifos degradation capability through organophosphate hydrolase (OPH) enzyme production, making it a superior candidate for bioremediation applications compared to all other isolated strains.

Table 3. Screening the growth tolerance of isolated bacteria by degrading Chlorpyifos

Organism code	25 µL	50 µL	75 µL	100 µL
AKCD01	+	-	-	-
AKCD02	-	-	-	-
AKCD03	+	-	-	-
AKCD04	+	+	+	-
AKCD05	+	+	+	-
AKCD06	+	+	+	+
AKCD07	+	+	+	+
AKCD08	+	+	+	-
AKCD09	-	-	-	-
AKCD10	+	+	+	+
AKCD11	+	+	+	-
AKCD12		+	+	+
AKCD13	-	-	-	-
AKCD14	+	+	-	-
AKCD15	+	-	-	-
AKCD16	+	-	-	-
AKCD17	+	+	-	-
AKCD18	+	+	+	-
AKCD19	+	+	+	+
AKCD20	+	+	+	+
AKCD21	+	-	-	-
AKCD22	+	-	-	-
AKCD23	-	-	-	-
AKCD24	-	-	-	-
AKCD25	+	-	-	-
AKCD26	+	+	+	-
AKCD27	-	-	-	-
AKCD28	-	-	-	-
AKCD29	+	-	-	-
AKCD30	+	-	-	-
AKCD31	+	+	+	-

Chlorpyrifos degradation screening of bacterial isolates

Screening of 31 isolated bacterial strains (AKCD01–AKCD31) for chlorpyrifos degradation at increasing concentrations (25 µL, 50 µL, 75 µL and 100 µL) revealed that five isolates AKCD06, AKCD07, AKCD10, AKCD19 and AKCD20 demonstrated robust growth at the highest concentration (100 µL), indicating exceptional organophosphate pesticide degradation (Table 3). Among these, AKCD07, AKCD10, AKCD12, AKCD19 and AKCD20 were identified as the most potential chlorpyrifos-degrading candidates based on consistent growth across all concentrations. AKCD12 showed unusual adaptive behavior (no growth at 25 µL but growth at higher concentrations). Notably, AKCA07 (*Pseudomonas aeruginosa*) from a separate isolate set exhibited superior potential beyond all other tested strains.

DISCUSSION

Significance of microbial degradation for soil bioremediation

Microbial degradation is an efficient method for removing chemical contamination from soil, with the ultimate goal of eliminating pollutants and creating agricultural soils suitable for organic crops (Shahid and Khan, 2022). Bioremediation exploits the microbial metabolism of native microorganisms and represents an ecological, cost-effective and highly efficient approach compared to physical and chemical methods (Shahid and Khan, 2022). Several biodegradation techniques are based on bacterial enzymatic degradation (Aldas-Vargas *et al.*, 2021).

Diversity and biotechnological potential of marine bacterial isolates

The diverse microbial community isolated from the three Kerala marine locations represents a valuable reservoir of marine bacteria with potential applications in bioremediation, enzyme production and biotechnological processes. *Bacillus* species (spore-formers resistant to environmental stress), *Pseudomonas* species (versatile metabolizers), *Vibrio* species (marine-adapted) and *Shewanella* species (metal-reducing capabilities) are particularly promising for further investigation into pesticide degradation, hydrocarbon breakdown and other environmental remediation applications (Muthukumaravel *et al.*, 2025). Reports exist on organophosphate (OP) pesticide-degrading potential for Bacillus cereus strains, including a recent study by Muthukumaravel *et al.* (2025).

Superior chlorpyrifos degradation capability of *Pseudomonas aeruginosa*

The identification of AKCA07 (*Pseudomonas aeruginosa*) is particularly significant. *P. aeruginosa* is well-documented as a highly efficient chlorpyrifos degrader possessing organophosphate hydrolase (OPH) enzymes that cleave the P–O bond in chlorpyrifos, converting it to the less toxic 3,5,6-trichloro-2-pyridinol (TCP) (Barragan-Huerta *et al.*, 2007). Shabbir *et al.* (2018) isolated *Pseudomonas aeruginosa*, *Enterobacter ludwigii* and *Enterobacter cloacae* from domestic wastewater to

test their ability to degrade chlorpyrifos, confirming *P. aeruginosa* as a superior candidate.

Comparison with previous studies on organophosphate pesticide degradation

Previous studies have shown that Serratia sp. and *Pseudomonas* sp. isolates completely degraded diazinon and malathion (initial concentration 50 mg/L) within 14 days (Cycon *et al.*, 2009; Massoud *et al.*, 2007). Various bacterial and fungal species have been reported to grow on diazinon, chlorpyrifos, or malathion, including *Serratia* sp. and *Pseudomonas* sp. (Cycon *et al.*, 2009). Barragan-Huerta *et al.* (2007) proposed a model for DDT biodegradation by bacteria grown in micro-niches created in porous coffee bean structures, identifying five bacterial species: *Pseudomonas aeruginosa*, *P. putida*, *Stenotrophomonas maltophilia*, *Flavimonas oryzihabitans* and *Morganella morganii*; in degradation of selected organochlorine compounds, *P. aeruginosa* showed better results.

Applications in field bioremediation and industrial biocatalysis

The high degradation capacity demonstrated by these isolates is a prerequisite for effective biodegradation, as microbes must survive in contaminated environments to metabolize toxins.

Their versatility supports applications in field bioremediation, bioaugmentation of contaminated sites and enzyme production for industrial biocatalysis (Lagiso *et al.*, 2026).

CONCLUSION

This study isolated and characterized 30 marine bacterial strains from Kerala marine waters (Cochin, Kollam and Calicut), representing diverse genera including *Bacillus, Pseudomonas, Vibrio, Serratia, Shewanella, Acinetobacter and Alteromonas*. Chlorpyrifos degradation screening identified five isolates (AKCD06, AKCD07, AKCD10, AKCD19, AKCD20) tolerant to 100 µL chlorpyrifos, with AKCD07, AKCD10, AKCD12, AKCD19 and AKCD20 as the most promising degraders. Notably, AKCA07 (*Pseudomonas aeruginosa*) showed superior degradation potential due to its organophosphate hydrolase (OPH) enzyme activity. These isolates, especially *P. aeruginosa* AKCA07, represent valuable candidates for bioremediation of chlorpyrifos-contaminated environments through bioaugmentation and enzyme production, warranting further investigation through quantitative degradation assays, enzyme characterization and field trials.

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