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Biodegradation of chlorpyrifos by soil bacteria and their effects on growth of rice seedlings under pesticide-contaminated soil

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Abstract: This study identified three soil bacteria (NRRU-BW3, NRRU-BW3, and NRRU-TV11) that degrade chlorpyrifos, produce indole-3-acetic acid, and exopolysaccharides under pesticide stress. The results revealed that soil bacteria were identified as *Priestia megaterium* NRRU-BW3, *Bacillus siamensis* NRRU-BW9, and *Bacillus amyloliquefaciens* NRRU-TV11. These strains showed the ability to produce indole-3-acetic acid (IAA) and exopolysaccharides (EPS) in chlorpyrifos. Moreover, these bacteria can degrade chlorpyrifos (CP) in an aqueous medium, and a 33–52% degradation rate was observed after 14 days of incubation. Inoculation with the NRRU-TV11 significantly increased ($P < 0.05$) plant height, root length, biomass and vigour index of rice seedlings compared to uninoculated controls in chlorpyrifos-contaminated soil. The findings demonstrated the beneficial effects of indigenous NRRU--TV11 on rice seedling development and chlorpyrifos degradation and recommended this strain as a potential replacement for plant growth improvement and environmental bioremediation of pesticide-contaminated agricultural soils.

Keywords: weed control; contamination; polluted soil; auxin; organophosphate; plant growth promoting bacteria

Agriculture frequently uses pesticides to control weeds, insect infestations, and diseases. Approximately 1% of the pesticide is applied to target pests; the remaining pesticide enters the soil and encounters several transformations, producing a complex pattern of metabolites. Pesticide residues can persist in the environment for various durations in atmospheric air, groundwater, surface water, soil, and sediments (Jara and Winter 2019). One of the most commonly used pesticides is an organophosphate, chlorpyrifos (CP) [O, O-diethyl O-(3,5,6-trichloro-2-pyridyl phosphorothioate)]. It has been widely utilised in domestic pest control and agricultural production to detect pesticides in food and the environment. Based on the soil type, climate, and other conditions, the half-life of CP in soil usually ranges from 60 to 120 days but can also be prolonged to over a year. A significant degradation by-product of CP, 3, 5, 6-trichloro-2-pyridinol (TCP) possesses antimicrobial characteristics and inhibits the growth of advantageous soil microbes (Supreeth et al. 2016). TCP adversely affects the diversity of the soil microbiota, reduces soil fertility, and inhibits plant growth, endangering the long-term viability of agricultural soils (Fang et al. 2009). It has a half-life of approximately 65–360 days. Diethyl thiophosphoric acid (DETP) is another by-product of CP that plays a crucial role in hormone-disrupting chemicals (Briceño et al. 2012). In addition, CP and its derivatives can also inhibit beneficial plant growth-promoting microorganisms in the soil (Akbar and Sultan 2016). In Thailand, several studies have

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investigated chlorpyrifos contamination in different environmental media. For example, a study of agricultural soils during winter reported the presence of chlorpyrifos in soil with concentrations of 28.57 ± 18.7 mg/kg (Harnpicharnchai et al. 2013). Another study investigated the presence of chlorpyrifos in water samples from the Chao Phraya River. The study found chlorpyrifos in all water with concentrations ranging from 0.01 to 13.40 µg/L (Wetchayanon et al. 2021).

Numerous reports have described various organophosphate pesticide treatments. Traditional cleaning methods for organophosphate pesticides rely on chemical reactions, recycling, pyrolysis, incineration, and landfill. However, this might result in the production of harmful compounds, which are both expensive and inefficient. On the other hand, the effective detoxification, degradation, and removal of harmful chemicals from polluted soil and water using microbes have emerged as successful methods to clean up polluted sites. Harmful organophosphate pesticides can be decomposed into less harmful derivatives using living organisms such as plants and microbes. In contaminated soil, many genera have been documented to break down organophosphate pesticides, including *Achromobacter*, *Ochrobactrum* (Akbar and Sultan 2016), *Arthrobacter*, *Bacillus*, *Microbacterium* (Ahirwar et al. 2019, Srinivasan et al. 2020), as well as *Alcaligenes* (Yadav et al. 2020). However, only some bacterial strains that can degrade CP and produce plant growth regulators have been documented.

Plant growth-promoting bacteria (PGPB) have attracted attention for encouraging plant growth and degrading environmental pesticides (Rani et al. 2019). They exhibit significant plant growth-promoting traits, such as phosphate solubilisation, indole-3-acetic acid (IAA) synthesis, and ammonia production, both in the absence and presence of CP, which are distinctive characteristics of CP degradation and plant growth promotion capabilities (Akbar and Sultan 2016). IAA, the main auxin in plants, is an essential plant hormone strongly implicated in plant growth and development. It is a crucial molecule that upregulates numerous genes involved in plant growth and development. It is a multi-functional plant hormone that regulates apical dominance, phototropic and geotropic responses, flower development, fruit ripening, cell division, elongation, and differentiation (Olatunji et al. 2017). Therefore, IAA-producing bacteria have attracted considerable attention in recent years. Bacterial exopolysaccharides (EPS), complex carbohydrates, play important roles in various biological processes, including cell protection, adhesion, and signalling. Some evidence suggests that EPS may have a role in mitigating the harmful effects and enhancing the biodegradation of chlorpyrifos in soil and water (Yadav et al. 2020). Therefore, IAA and EPS production during chlorpyrifos degradation by microorganisms can be advantageous in multiple ways.

The utilisation of bacteria in degrading chlorpyrifos in rice fields in Thailand is an important approach to address the issue of pesticide contamination. Chlorpyrifos is a highly toxic pesticide commonly used in rice farming, which can have negative impacts on human health and the environment. Using bacteria to degrade chlorpyrifos can reduce its harmful effects, promoting sustainable agriculture practices. Bacteria can break down chlorpyrifos into less toxic compounds, which can then be safely absorbed by the soil. This not only reduces the risk of pesticide exposure for farmers and consumers but also helps to maintain the health and biodiversity of soil ecosystems. Moreover, utilising bacteria in chlorpyrifos degradation is a cost-effective and environmentally friendly method compared to other conventional methods such as chemical or physical treatments. It is also a scalable and adaptable approach that can be applied in various farming systems and regions. Therefore, the utilisation of bacteria in degrading chlorpyrifos in rice fields in Thailand is a crucial step towards promoting sustainable agriculture and protecting human health and the environment. Previously, three strains of soil bacteria were isolated from the agricultural soils of Nakhon Ratchasima province, Thailand. These sites have been treated with pesticides for a long time (> 10 years). These bacteria were isolated and screened as potent CP-resistant bacteria (Saengsanga et al. 2018). Consequently, this study was initiated to assess the potential of indigenous soil bacteria (NRRU-BW3, NRRU-BW9, and NRRU-TV-11) to destroy CP in a culture medium and CP-amended soil and their correlation with IAA and EPS production. Likewise, the effect of these bacteria on alleviating chlorpyrifos toxicity in rice was evaluated.

MATERIAL AND METHODS

Chemicals and reagents. Analytical grade chlorpyrifos (99.61%, *w*/*v*) purchased from Dr Ehrenstorfer

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GmbH (Augsburg, Germany) was used as the standard. Commercial grade chlorpyrifos (40%, *w*/*v*) (G.I. fos) was purchased from a local market in Nakhon Ratchasima province, Thailand. All other chemicals were purchased from Sigma-Aldrich, Loba Chemie, or BDH. CP stock solution (1 000 mg/L) was dissolved in acetone.

Bacterial strains and media. Bacteria used in this study were NRRU-BW3, NRRU-BW9 and NRRU-TV11 (Saengsanga et al. 2018), which were previously isolated from pesticide-contaminated soil in Nakhon Ratchasima province, Thailand, where treated extensively with pesticides. Briefly, 10 g of each soil was dissolved in 90 mL of sterile normal saline solution (0.85% *w*/*v* NaCl) in a 250-mL Erlenmeyer flask. The soil suspension was shaken at 150 rpm for 30 min at 30 °C. 1 mL of suspension was serially diluted to obtain a solution with 10^{-3}–10^{-5} dilution, and the obtained solution was subjected to plated on mineral salt medium (MSM) (1.5 g/L K_2HPO_4, 0.5 g/L KH_2PO_4, 0.2 g/L $MgSO_4 \cdot 7\ H_2O$, 0.5 g /L NaCl, and 1.5 g/L NH_4NO_3) supplemented with 50 mg/L of chlorpyrifos (commercially available, G.I. FOS). Plates were incubated at 30 °C for 24 h; the tolerant strains were then selected for further determination.

Identification of bacteria by 16S rRNA sequences. Chromosomal DNA was extracted using a DNeasy Power Soil Pro Kit (Qiagen, Germany). Amplification of the 16S-rRNA gene was performed in a 30 µL reaction mixture consisting of 1X EF-Taq Reaction Buffer (2.5 mmol $MgCl_2$ mixed), 1.0 mmol dNTP Mix, 20 ng DNA template and 0.5 µmol 27-F and 1492-R. The sequences of the primer pairs used were 27-F (5'-AGAGTTTGATCMTGGCTCAG-3') and 1492-R (5'-TACGGYTACCTTGTTACGACTT-3'). The PCR conditions were done as follows: pre-denaturation at 95 °C for 2 min; 35 cycles of 95 °C for 1 min, 55 °C, and 72 °C for 1 min each were performed, and future incubation at 72 °C for 10 min. The PCR products were purified and bidirectionally sequenced by Macrogen (Seoul, South Korea). The retrieved sequences were matched with those of the 16S-rRNA sequences provided in GenBank using the BLAST program (http://blast.ncbi. nlm.nih.gov/Blast.cgi). Multiple alignments of the related sequences were performed using ClustalX 2.0 (Larkin et al. 2007). A phylogenetic tree was constructed in MEGA11 software (Tamura et al. 2021) using the Maximum Likelihood method and the Jukes-Cantor model (Jukes and Cantor 1969). A bootstrap consensus tree with 1 000 replicates was constructed. The 16S-rRNA sequences were deposited in the GenBank database, and the accession numbers are listed in Table 1.

IAA and EPS production by bacteria. IAA production was determined by Salkowski's colourimetric assay (Hassen et al. 2018). Briefly, 20 mL of MSM was inoculated with 2% (*v*/*v*) cell suspension (OD600 = 1) and incubated at 30 °C and 150 rpm for 24 h. The culture medium was supplemented with 10, 20, and 30 mg/L of CP, and no additional CP served as the control. The test was conducted in 3 replicates. The cell suspension was centrifuged (Hettich MIKRO 220R, Tuttlingen, Germany), and 2 mL of the supernatant was mixed with 100 µL of orthophosphoric acid and 4 mL of Salkowski's reagent (100 mL of 35% (*v*/*v*) perchloric acid with 2 mL of 0.5 mol/L iron (III) chloride). The reaction was kept in the dark for 1 h and subjected to quantify IAA concentration by spectrophotometry at 535 nm (Evolution 600 UV-Vis Spectrophotometer, Waltham, USA). IAA concentration in the supernatant was calculated using the IAA standard curve.

EPS production by bacteria was determined during growth in batch culture using MSM supplemented with 10, 20, and 30 mg/L CP, and no additional CP served as the control. An overnight starter culture was prepared, inoculated, and agitated on a rotary shaker (150 rpm) at 30 °C for 72 h. Samples were taken at 24 h intervals to evaluate growth and EPS production. The growth of the bacterial cultures was measured at OD600 (optimal density) by spectrophotometry. The cell-free supernatant was collected for soluble EPS quantification and precipitated with 2 volumes of pre-chilled acetone. This mixture was stored at 4 °C overnight before centrifugation at 12 000 rpm at 4 °C for 20 min. The pre-precipitated

Table 1. Identification of bacteria by 16S-rRNA sequence analysis

Strains	Accession number	Bacterial identification
NRRU-BW3	ON248946	*Priestia megaterium*
NRRU-BW9	ON248947	*Bacillus siamensis*
NRRU-TV11	ON248948	*Bacillus amyloliquefaciens*

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EPS was dissolved in distilled water and added to a 5% (*w*/*v*) phenol solution. The reaction mixture was added to 5 mL of concentrated sulfuric acid, and the optical density was determined at 490 nm (Yadav et al. 2020). The presence of EPS was calculated from the calibration curve using glucose as the standard.

Growth determination and rapid detection of CP degradation in liquid medium. CP degradation was detected using the UV spectrophotometric method described by Zalat et al. (2014). Briefly, 5% (*v*/*v*) overnight starter culture was inoculated in 50 mL of MSM. The medium was supplemented with 10 mg/L CP, and an uninoculated flask was used as a control. The flask was maintained at 30 °C for 12 consecutive days, and samples were taken every two days to examine growth and CP degradation. Growth was determined by measuring OD at 600 nm. The residual of CP was detected by harvesting 1.5 mL of culture medium and centrifuging at 4 °C at 10 000 rpm for 10 min. The pellet was discarded, and the cell-free supernatant was analysed using spectrophotometry at A290 nm. The residues of CP were calculated from the calibration curve using analytical grade CP as the standard. The % residue was calculated according to Eq. 1:

$$\text{CP biodegradation (\%)} = (\text{Cpi} - \text{CPf})/\text{CPi} \times 100 \quad (1)$$

Where: CPi – initial CP concentrations; CPf – final CP concentrations.

Effects of bacterial inoculation on growth of rice seedlings growth in pesticide-contaminated soil. The experiment was carried out in a completely randomised design (CRD) with different 5 treatments under normal conditions (control) and CP-contaminated soil (uninoculated, inoculated with NRRU-BW3, NRRU-BW9, NRRU-TV11). Healthy rice seeds (*Oryza sativa* L. cv. KDML105) were selected and disinfected according to Oyebanji et al. (2009). Sterilised rice seeds were immersed in each bacterial suspension for 12 h. Bacterised seeds (20 seeds/replicate) were germinated and irrigated with 15 mL of 100 mg/L CP along with sterile distilled water as a control. The germination percentage was recorded after 4 days of sowing. Germinated rice seeds (5 plants/replicates) were transplanted into pots containing 100 mg/kg CP-contaminated soil. Fifteen days after transplanting (DAT), rice growth performance was analysed using plant elongation assays, biomass analysis, and vigour index. Vernier callipers were used to measure plant elongation. Biomass was analysed by weighing using a digital balance with 0.0001 g accuracy (Denver Instrument, New York, USA). The vigour index of rice seedlings was computed using Eq. 2:

$$\text{Vigor index} = \text{\% Germination} \times \times (\text{plant height} + \text{root length}) \quad (2)$$

Data analysis. Experiments were carried out in 3 replicates, and statistical data are presented as the mean ± standard deviation (SD). Analysis of variance (ANOVA) was used to determine the significant differences between the means by Duncan's multiple range test (DMRT). The significance level of $P < 0.05$ was used to examine the data for homogeneity of variances.

RESULTS

Identification of bacteria. Colonies of the strains NRRU-BW3, NRRU-BW9, and NRRU-TV11 on nutrient agar plates and phylogenetic trees related to other species are shown in Figure 1. The alignment results of the 16S-rRNA sequences retrieved from the Basic Local Alignment Search Tool (BLAST) in the NCBI database revealed that the strains NRRU-BW3 were 99.93% similar to those of *Bacillus megaterium* VIT-SRM2 (KJ716459), NRRU-BW9 was 99.34% similar to *B. siamensis* S5 (MZ148821), and NRRU-TV11 was 99.86% similar to *B. amyloliquefaciens* 205 (CP054415). Consequently, the isolates were identified as *Priestia megaterium* NRRU-BW3 (formerly known as *B. megaterium*), *B. siamensis* NRRU-BW9, and *B. amyloliquefaciens* NRRU-TV11. However, a phylogenetic tree revealed that isolates NRRU-BW9 and NRRU-TV11 might belong to similar species based on the dendrogram branch.

IAA and EPS production by bacteria. IAA concentration was examined in the presence and absence of CP (0, 10, 20, and 30 mg/L) in an IAA medium with 1 g/L of L-tryptophan as a precursor of the IAA biosynthesis. As shown in Figure 2, the IAA concentration produced by NRRU-BW3 ranged from 6–13 mg/L, whereas that of NRRU-BW9 ranged from 5–10 mg/L and NRRU-TV11 ranged from 11–32 mg/L. All bacterial strains cultured without CP exhibited high levels of IAA, which were 32, 13, and 10 mg/L for NRRU-TV11, NRRU-BW3, and NRRU-BW9, respectively.

The concentration of EPS secreted by the strains decreased when the concentration of CP increased (Figure 3). The highest concentrations in isolate NRRU-BW3, NRRU-BW9, and NRRU-TV11 were 3.66, 4.85, and 6.54 mg/L at a 48 h incubation pe-

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(A)

(B)

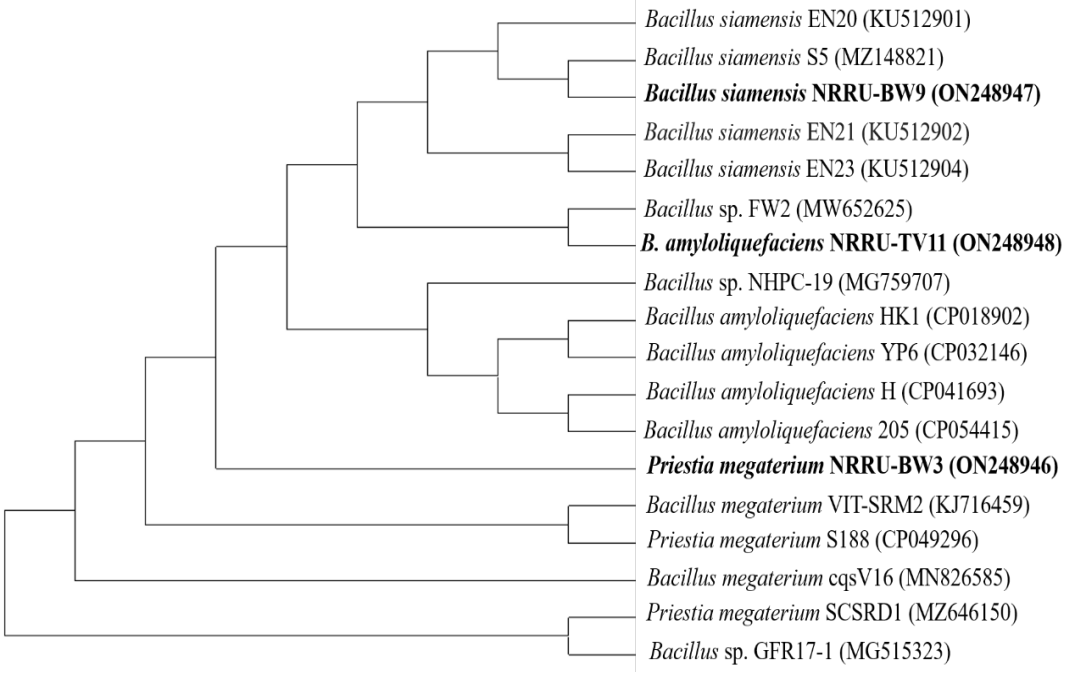

Figure 1. (A) Bacterial colony of NRRU-BW3, NRRU-BW9 and NRRU-TV11 bacteria growing on a nutrient agar plate, and (B) a phylogenetic tree based on 16S-rRNA gene sequences, showing the phylogenetic relationship of the three strains with strains of closely related genera

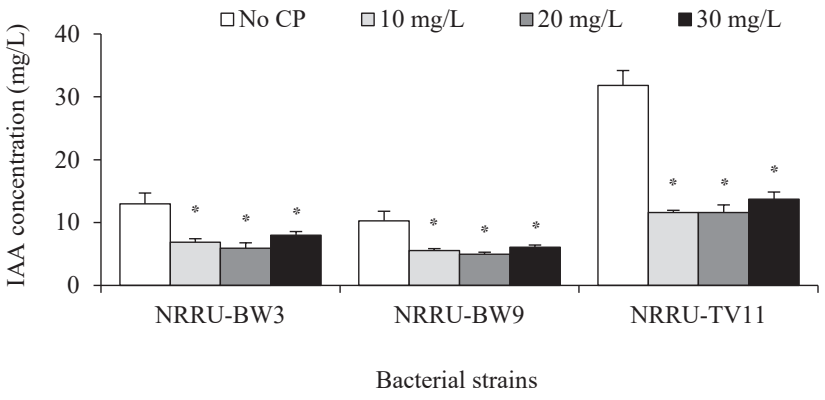

Figure 2. Indole-3-acetic acid (IAA) production by the NRRU-BW3, NRRU-BW9, and NRRU-TV11 in mineral salt medium (MSM) containing L-tryptophan (1 g/L) and different concentrations of chlorpyrifos (CP) (10, 20, and 30 mg/L) compared with control without CP (no CP). Values are means ± standard deviation of three replicates, with stars (*) indicating statistically significant differences between treatments of each bacterial strain at $P < 0.05$ (Duncan's multiple range test)

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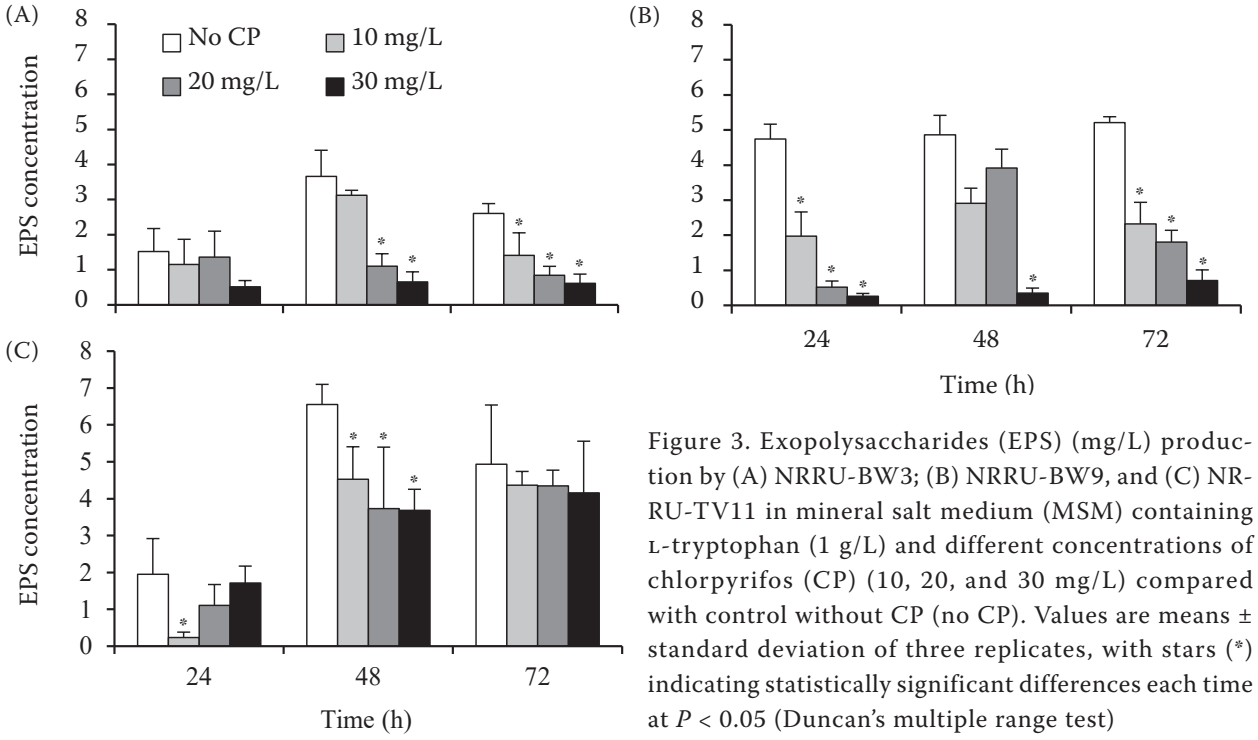

Figure 3. Exopolysaccharides (EPS) (mg/L) production by (A) NRRU-BW3; (B) NRRU-BW9, and (C) NRRU-TV11 in mineral salt medium (MSM) containing L-tryptophan (1 g/L) and different concentrations of chlorpyrifos (CP) (10, 20, and 30 mg/L) compared with control without CP (no CP). Values are means ± standard deviation of three replicates, with stars (*) indicating statistically significant differences each time at $P < 0.05$ (Duncan's multiple range test)

riod without CP, respectively. For NRRU-BW3, EPS production gradually decreased when bacteria were exposed to CP after 24 h of incubation. As shown in Figure 3A, after the 48 h incubation period, EPS production by NRRU-BW3 was significantly inhibited by CP at a concentration of 20–30 mg/L. Moreover, EPS

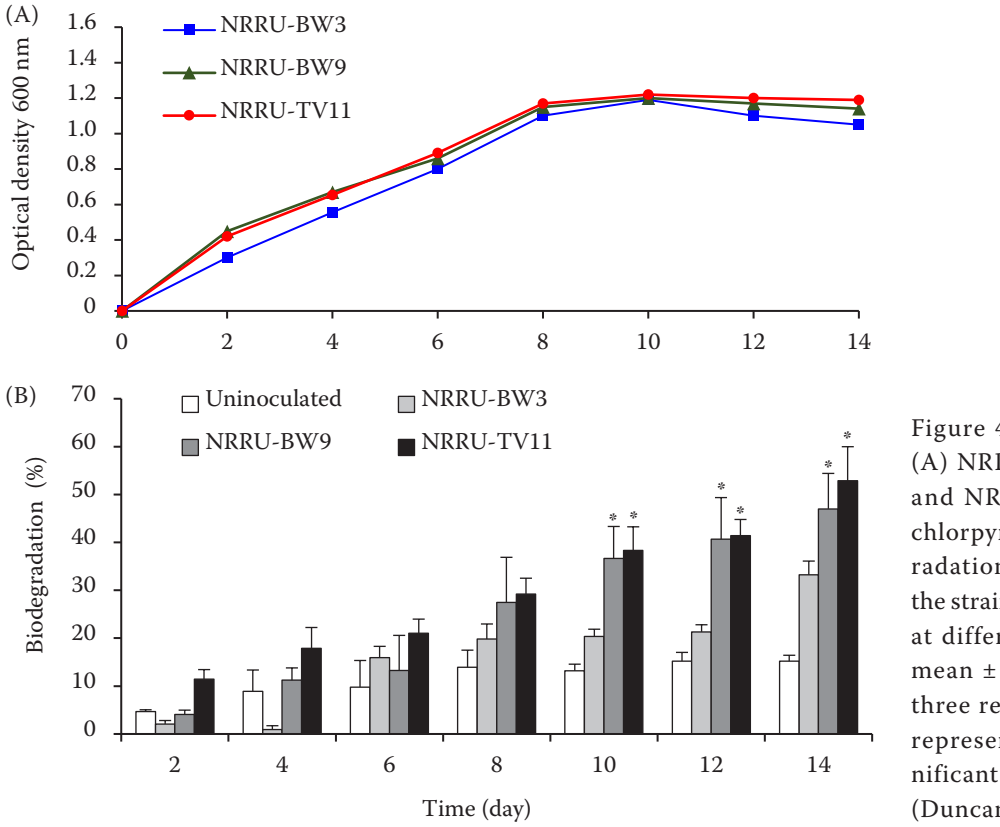

Figure 4. Growth patterns of (A) NRRU-BW3, NRRU-BW9 and NRRU-TV11 containing chlorpyrifos (CP) and (B) degradation percentage of CP by the strains in the liquid medium at different times. Values are mean ± standard deviation of three replicates, with stars (*) representing statistically significant differences at $P < 0.05$ (Duncan's multiple range test)

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production by NRRU-BW9 was effectively restrained after 24 h with 10 mg/L CP (Figure 3B). In the case of NRRU-TV11, EPS production significantly decreased after 48 h of incubation with CP (Figure 3C).

Bacterial growth and CP degradation. The growth of the three bacterial strains was observed in MSM supplemented with CP. The results found that eight days of incubation resulted in active growth without an initial lag phase. Slower growth was detected between eight and ten days and then entered the stationary phase after ten days of incubation (Figure 4A). The residue of CP by the strains was proportional to bacterial growth and time. As shown in Figure 4B, all the strains could degrade CP in the MSM. After two days of incubation, CP initiated degradation. Among these, NRRU-TV11 was found to be highly effective in degrading CP. The CP biodegradation of the NRRU-TV11 was about 52%, followed by NRRU-BW9 (47%) and NRRU-BW3 (33%) within 14 days of incubation.

Effects of the soil bacteria on growth of rice seedlings in CP-contaminated soil. Plant growth experiments were used to investigate the effects of the strains on reducing CP toxicity in rice by analysing growth performance parameters. In the presence of CP, it was observed that rice with CP had reduced growth parameters compared to rice without CP (Figure 5). Seed germination with 100 mg/L CP supplementation inoculated with the strains was not significantly different ($P > 0.05$). However, all strains significantly improved ($P < 0.05$) rice seedlings' total length and vigour index compared to uninoculated controls. Among the CP treatments, isolate NRRU-TV11 stimulated the highest total length (44.8 cm) and vigour index (2 870), corresponding to a 32% and 31% increase, respectively, compared to uninoculated rice seedlings (Table 2). An increase of 30.1, 21.6 and 33.85% in plant height and 7.1, 18.1, and 26.2% in root length were recorded in rice seedlings inoculated with NRRU-BW3, NRRU-BW9, and NRRU-TV11, respectively compared with uninoculated controls (Figure 5A, B). Biomass was determined based on the dry weight of shoots and roots (Figure 5C). Among the CP treatments, the average increase in biomass was 51.8% for rice inoculated with NRRU-TV11, followed by NRRU-BW9 with an increase of 28.7% when compared to uninoculated controls. Consistent with our previous experiments, NRRU-TV11 was a potent isolate that could encourage plant growth and alleviate CP stress. Increments in elongation and biomass of rice indicate that these bacteria not only display the ability to degrade CP but also protect and promote the growth of rice against stress from CP *via* IAA and EPS secretion.

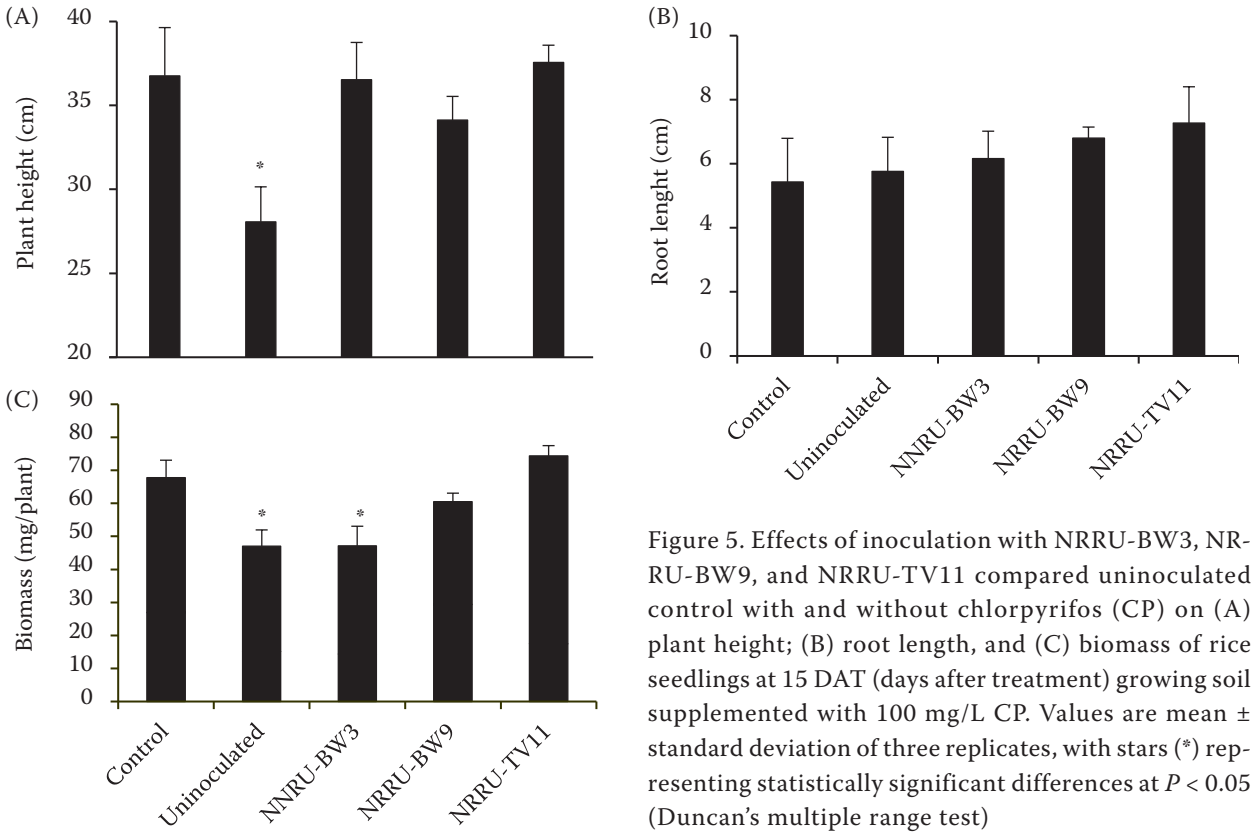

Figure 5. Effects of inoculation with NRRU-BW3, NRRU-BW9, and NRRU-TV11 compared uninoculated control with and without chlorpyrifos (CP) on (A) plant height; (B) root length, and (C) biomass of rice seedlings at 15 DAT (days after treatment) growing soil supplemented with 100 mg/L CP. Values are mean ± standard deviation of three replicates, with stars (*) representing statistically significant differences at $P < 0.05$ (Duncan's multiple range test)

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Table 2. Effects of the strains on % germination, total lengths and vigour index of rice seedlings at 15 DAT (days after treatment) growing in soil supplemented with 100 mg/L chlorpyrifos (CP)

Condition	Treatment	% Germination	Total lengths (cm)	Vigour index
Normal	control	86.7 ± 2.3	42.2 ± 1.8	3 656 ± 132
CP-contaminated soil	uninoculated	64.0 ± 4.0*	33.8 ± 2.8*	2 178 ± 428*
	NNRU-BW3	62.7 ± 2.3*	42.7 ± 3.0	2 645 ± 851*
	NRRU-BW9	60.0 ± 6.93*	40.9 ± 1.1	2 506 ± 189*
	NRRU-TV11	64.0 ± 4.0*	44.8 ± 1.0	2 870 ± 205

Values are mean ± standard deviation of three replicates with stars (*) representing statistically significant differences in each column at $P < 0.05$ (Duncan's multiple range test)

DISCUSSION

Recently, the degradation of chlorpyrifos by bacteria has gained significant attention due to its potential as a sustainable and eco-friendly method of reducing pesticide contamination in the environment. Several bacterial species have been identified to have the ability to degrade chlorpyrifos through various metabolic pathways. Feng et al. (2017) explored the diversity of endophytic bacteria isolated from CP-contaminated rice plants by 16S rDNA sequence analysis.

They identified five species of endophytic bacteria: *B. megaterium* RRB, *Curtobacterium plantarum* RSC24, *Pseudomonas aeruginosa* RRA, *Sphingobacterium siyangensis* RSA, and *Stenotrophomonas pavanii* RSB. In addition, the common free-living soil bacteria isolated from the pesticide-polluted sites were *Achromobacter xylosoxidans* (JCp4), *Ochrobactrum* sp. (FCp1) (Akbar and Sultan 2016), *B. safenensis* FO-36bT, *B. subtilis* subsp. *inaquosorum* KCTC13429T, *B. cereus* ATCC14579T (Ishag et al. 2017), *B. pumilus* C2A1 (Anwar et al. 2009), and *Bacillus* sp. (Srinivasan et al. 2020). Although none of the three strains in this study belonged to distinct genera, several interesting characteristics require further investigation. *Bacillus* species are one of the most common bacterial genera found in soil and are recognised worldwide as being generally recognised safe (GRAS) microorganisms (Saxena et al. 2020). Consequently, *P. megaterium* NRRU-BW3, *B. siamensis* NRRU-BW9, and *B. amyloliquefaciens* NRRU-TV11 are not considered pathogenic to humans, animals, plants, and environments.

IAA and EPS are two important components produced by bacteria that play critical roles in their growth and survival. IAA is a plant hormone produced by some bacteria that helps to promote plant growth and development. It acts as a growth regulator, promoting root elongation and inducing lateral root formation. IAA production has been observed in a wide range of PGPB (Olatunji et al. 2017). EPS, on the other hand, is a complex mixture of polymers secreted by bacteria into their surrounding environment. EPS plays several crucial roles in bacterial physiology, such as protecting bacteria from environmental stresses, aiding nutrient acquisition, and promoting biofilm formation and plant-microbe interaction (Lu et al. 2020). The production of IAA and EPS by these bacteria during chlorpyrifos degradation can be beneficial in several ways. However, the amount of IAA was dramatically reduced (2–3 folds) as the concentration of CP increased, indicating that CP has adverse effects on the IAA biosynthesis pathway. According to Fang et al. (2009), the plant growth-promoting ability of bacteria is significantly decreased in the presence of CP. It is recognised that they have adverse effects on inhibiting the activities of soil bacteria. Similarly, the plant growth-promoting traits of beneficial soil bacteria decreased in pesticide concentrations exceeding the recommended doses (Madhaiyan et al. 2006). Therefore, pesticides may cause a decrease in IAA and EPS production by microorganisms (Sultana et al. 2019). This study indicates that the amounts of EPS produced by all strains tested were inhibited by CP toxicity. A similar study by Lu et al. (2020) indicated that CP inhibits the production and release of EPS by suppressing four EPS synthesis-related genes in *Pseudomonas stutzeri* A1501. Furthermore, xenobiotics induce excessive reactive oxygen species (ROS), which degrade intracellular and extracellular polysaccharides (Yu et al. 2020). However, the ability of bacteria to degrade chlorpyrifos while simultaneously producing IAA and EPS highlights the potential for sustainable and eco-friendly approaches to pest management in agriculture.

For CP biodegradation, the study has shown that bacteria-mediated degradation of chlorpyrifos can

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effectively reduce its concentration in a liquid medium. This result agreed with the report by several studies on CP degraders such as *Bacillus pumilus* C2A1, which destroys approximately 70% of CP in MSM within 3 days of incubation (Anwar et al. 2009). *Bacillus safensis* FO-36bT, *B. subtilis* subsp. *inaquosorum* KCTC 13429T, and *B. cereus* ATCC14579T could remove CP from MSM by approximately 89, 89, and 87%, respectively, of 400 mg/L CP in 30 days (Ishag et al. 2017). The biodegradation rate of CP by three isolates of *Bacillus* spp. removed 52% within ten days (Maya et al. 2011). Liu et al. (2012) reported that *B. cereus* could degrade up to 74% of its initial 100 mg/L concentration. In addition, *Bacillus* sp. AGM5 was recently described as a malathion degrader, utilising 57% within seven days (Dar and Kaushik 2022). Furthermore, bacterial consortia, including *Agrobacterium tumefaciens* ECO1, *Cellulosimicrobium funkei* ECO2, *Shinella zoogloeoides* ECO3, and *Bacillus aryabhattai* ECO4, degraded 100% of CP (50 mg/L) within 6 days (Uniyal et al. 2021). The biodegradation of CP by bacteria depends on the culture conditions, inoculum size, and bacterial species. However, by adding other nutrients for co-metabolisms, CP degradation is greatly accelerated due to high growth in easily metabolisable compounds, which increases degradation (Anwar et al. 2009). The degradation of chlorpyrifos by bacteria is a promising approach towards reducing the negative impact of pesticides on the environment.

Various pesticides have been reported to have adverse effects, such as disrupting the development of reproductive organs and reducing growth. Phytotoxicity has been reported in sweet pepper (Giménez-Moolhuyzen et al. 2020), radish, and green grams. The pesticide lindane inhibited germination in green gram and radish at 100 g/L (Bidlan et al. 2004). Previously, *Pseudomonas rhizophila* S211 was recognised as a PGPB with biofertilisation, biocontrol, and bioremediation capabilities (Hassen et al. 2018). Usually, bacteria isolated from the rhizosphere or agricultural soil have PGP traits. Some of these bacteria may degrade pesticides. *Achromobacter xylosoxidans* (JCp4) and *Ochrobactrum* sp. (FCp1) have the potential for chlorpyrifos degradation. They can produce IAA, and phosphate solubilise (Akbar and Sultan 2016). Several *Bacillus* species, including *Bacillus pumilus* C2A1 (Ahmad et al. 2012), *Bacillus flexus* (Kaur et al. 2022), and *Bacillus* sp. GL5, *Bacillus* sp. H1-80 (Slimani et al. 2022) has been documented as a plant growth-promoting bacteria. Based on their properties, PGPB can degrade CP, protect and promote plant growth, and enhance bioremediation and phytoremediation processes. Therefore, the positive correlation between plant growth-promoting traits and CP degradation by NRRU-BW3, NRRU-BW9, and NRRU-TV11 could improve plant growth and health in contaminated soil. The enhanced plant growth performance could be attributed to IAA and EPS production and CP degradation, resulting in improved plant growth in polluted soil. However, soil conditions can significantly impact the behaviour and survival of the bacteria, particularly in the presence of various other factors such as high concentrations of pollutants, other pesticides, and bacteria. Soil pH, nutrient levels, organic matter content, moisture levels, inoculum sizes, and rotations can all affect bacterial growth and survival (Dar and Kaushik 2022). Bacteria-mediated chlorpyrifos breakdown in soil reduces its risk of harming humans and the environment. This approach is cost-effective and adaptable to various agricultural systems. However, further research is needed to optimise the conditions for the degradation process and develop strategies for large-scale applications.

In conclusion, this study demonstrated the ecological consequences of CP and elucidated its adverse effects on plant growth and bacterial populations. These bacteria were identified as *P. megaterium* NRRU-BW3, *B. siamensis* NRRU-BW9, and *B. amyloliquefaciens* NRRU-TV11. Our research provides information on the ecological effects of CP on soil bacteria and emphasises its detrimental effects on the early growth of rice plants. The findings demonstrated that all of the bacteria tested had a favourable impact on the degradation of CP and the growth of rice seedlings, with the isolate NRRU-TV11 having the highest performance. It suggests that these bacteria produced IAA and EPS, aiding in improving plant growth under pesticide-contaminated soil. More research is being done to assess how this bacterium interacts with the rice plant in the field.

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