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Soil bacteria showing a potential of chlorpyrifos degradation and plant growth enhancement

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ABSTRACT

Background: Since 1960s, the organophosphate pesticide chlorpyrifos has been widely used for the purpose of pest control. However, given its persistence and toxicity towards life forms, the elimination of chlorpyrifos from contaminated sites has become an urgent issue. For this process bioremediation is the method of choice.

Results: Two bacterial strains, JCp4 and FCp1, exhibiting chlorpyrifos-degradation potential were isolated from pesticide contaminated agricultural fields. These isolates were able to degrade 84.4% and 78.6% of the initial concentration of chlorpyrifos (100 mg L^{-1}) within a period of only 10 days. Based on 16S rRNA sequence analysis, these strains were identified as *Achromobacter xylosoxidans* (JCp4) and *Ochrobactrum* sp. (FCp1). These strains exhibited the ability to degrade chlorpyrifos in sterilized as well as non-sterilized soils, and were able to degrade 93–100% of the input concentration (200 mg kg^{-1}) within 42 days. The rate of degradation in inoculated soils ranged from 4.40 to 4.76 mg $^{-1}$ kg^{-1} d^{-1} with rate constants varying between 0.047 and 0.069 d^{-1}. These strains also displayed substantial plant growth promoting traits such as phosphate solubilization, indole acetic acid production and ammonia production both in absence as well as in the presence of chlorpyrifos. However, presence of chlorpyrifos (100 and 200 mg L^{-1}) was found to have a negative effect on indole acetic acid production and phosphate solubilization with percentage reduction values ranging between 2.65–10.6% and 4.5–17.6%, respectively. Plant growth experiment demonstrated that chlorpyrifos has a negative effect on plant growth and causes a decrease in parameters such as percentage germination, plant height and biomass. Inoculation of soil with chlorpyrifos-degrading strains was found to enhance plant growth significantly in terms of plant length and weight. Moreover, it was noted that these strains degraded chlorpyrifos at an increased rate (5.69 mg $^{-1}$ kg^{-1} d^{-1}) in planted soil.

Conclusion: The results of this study clearly demonstrate that the chlorpyrifos-degrading strains have the potential to develop into promising candidates for raising the productivity of crops in pesticide contaminated soils.

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Introduction

The extensive use of pesticides through field application, crop spraying, handling, rinsing of containers, accidental spills, etc. has a potential to severely contaminate soil.[1] Most of the pesticides that are in common usage today are known to adversely affect functional diversity of the soil microbiota leading to loss of soil fertility and plant growth, which in turn put the sustainability of agricultural soil at serious risk.[2] To add to the complexity of the situation, pesticide residues and their metabolites often infiltrate through the soil surface into the groundwater and cause widespread contamination of aquatic ecosystems.[3]

Chlorpyrifos (CP) is a broad spectrum organophosphate insecticide that is classified as moderately toxic. Since 1960s, CP has been extensively used in the agricultural sector for controlling insect infestations of crops such as cotton, cereals, vegetables and fruits.[4] Although CP is considered only moderately toxic, it is known to possess neurotoxic and immunotoxic properties and has been shown to be harmful to both animals and humans.[5] CP has also been reported to cause a reduction in the bacterial, fungal and actinomycete population of the soil[6] and is known to inhibit nitrogen mineralization in soil.[7] Detection of CP contamination in surface water bodies and associated sediments has heightened public concern on the topic[8,9] and warranted urgent attention and treatment of the problem.

Bioremediation is a method that exploits the potential of microbial degradation for providing a cost-effective and reliable approach to pesticide abatement. Several soil and aquatic environments have been successfully reclaimed from pesticide contamination by using microbes capable of degrading the pollutants.[10] Hydrolysis, either chemically or as a result of microbial activity, degrades CP by converting it to diethylthiophosphoric acid (DETP) and 3,5,6-trichloro-2-pyridinol (TCP).[11] Pesticide degrading bacteria found in soil are known to have multifarious abilities such as phytohormone production, mineral solubilization, N_2-fixation, etc., which are extremely crucial for promotion of plant growth. Presence of the above-mentioned traits underlines and emphasizes the agronomic and environmental significance of such microbes. The potential of microbes to simultaneously detoxify pollutants while enhancing plant growth has been studied previously for pesticides carbofuran and thiamethoxam.[12,13]

The objective of this study was to isolate and characterize CP-degrading bacteria and to determine the degradation potential of these strains in both sterile as well as non-sterile soil. Moreover, plant growth promoting potential of these bacteria was also assessed; and the strains were tested for their ability to bioremediate soil and enhance plant growth in contaminated soil.

Materials and methods

Chemicals and media

Technical grade chlorpyrifos (98%) was obtained from the Ali Akbar group, Pakistan. Analytical grade CP and 3,5,6-trichloro-2-pyridinol (TCP) were purchased from Sigma–Aldrich (St Louis, MO, USA). HPLC grade organic solvents were procured from Merck (Pakistan). Microbial isolations were conducted in a mineral salt medium (MSM) (pH 6.8–7.0) containing ($g\,L^{-1}$) K_2HPO_4, 1.5; KH_2PO_4, 0.5; NaCl, 0.5; $(NH_4)_2SO_4$, 0.5; $MgSO_4{\cdot}7H_2O$, 0.2, and 10 mL of 100× trace element solution. The 100× trace element solution was composed of ($mg\,L^{-1}$) $Na_2EDTA{\cdot}2H_2O$, 500; $FeCl_2{\cdot}4H_2O$, 143; $ZnCl_2$, 4.7; $MnCl_2{\cdot}4H_2O$, 3.0; H_3BO_3, 30; $CoCl_2{\cdot}6H_2O$, 20; $CuCl_2{\cdot}2H_2O$, 1.0; $NiCl_2{\cdot}6H_2O$, 2.0; $Na_2MoO_4{\cdot}2H_2O$, 3.0; and $CaCl_2{\cdot}2H_2O$, 100.

Enrichment, selection and identification of chlorpyrifos degrading strains

Soil samples from agricultural fields of Jhang and Faisalabad, Punjab, Pakistan, were used for the purpose of enrichment of chlorpyrifos degrading bacteria. Five grams of soil was added to Erlenmeyer flasks (250 mL) containing 50 mL MSM supplemented with 50 $mg\,L^{-1}$ CP and the culture was incubated at 150 rpm, 30 °C. The enrichment process was conducted over a period of four weeks; following an established protocol,[14] at regular intervals of one week each of the culture was transferred into fresh growth medium. Subsequent to the last enrichment process, serial dilutions of the culture were spread on MSM plates containing CP (50 $mg\,L^{-1}$) and the colonies thus isolated were further purified by the streak plate method.

For determining the degradation potential, cultures were grown in triplicate in MSM containing 100 $mg\,L^{-1}$ CP for a period of 10 days and the residual CP concentration was determined by high performance liquid chromatography (HPLC). CP quantification was analyzed on a SYKAM HPLC (Germany) using an S1122 HPLC Pump, S3210 UV detector, S1122 delivery system and Phenomenex C18 reversed-phase column (150 mm). Detector output was processed by the clarity chromatography data system. Samples were eluted using methanol:H_2O:acetic acid (80:20:0.5, v/v) at a flow rate of 1.0 $mL\,min^{-1}$. Detection was performed at 230 nm and the retention times of CP and TCP were determined to be 18.4 and 16.1 min, respectively. Two isolates with the highest CP degradation potential, designated as JCp4 and FCp1, were selected for further study.

Bacterial strains were identified by amplification of the 16S rRNA gene using universal primers 27f and 1492R. Amplification was carried out in a 50 μL reaction mixture containing 20 pmol of primer (F and R) each, 25 μL PCR Master Mix (Thermoscientific) and 20 ng of template DNA. The PCR thermocycling parameters were as follows: initial denaturation at 94 °C for 5 min; followed by 25 cycles of 94 °C (1 min), 50 °C (1 min); 72 °C (1 min); and final extension at 72 °C for 10 min. The amplified PCR products were sequenced and sequence homologies were identified using nBLAST. GenBank accession numbers were assigned for 16S rRNA gene sequences of both the isolates (KJ009240 and KJ009242).

Determination of auxiliary characteristics

Indole acetic acid (IAA) production

LB-broth (50 mL), supplemented with 50, 100 and 150 $\mu g\,mL^{-1}$ tryptophan, was inoculated with 50 μL of cell suspension ($OD_{600} = 0.5$) in triplicate and incubated at 35 °C, 120 rpm for