BIODEGRADATION OF THE CHLORPYRIFOS PESTICIDE BY BACTERIA ISOLATED FROM GROUNDNUT AGRICULTURAL SOILS IN KADAPA BASIN

Y. JAYASRI1,*, M. DHANANJAYA NAIDU1 AND M. MALLLKARJUNA2
1Department of Zoology, Yogi Vemana University, Kadapa - 516 003, Andhra Pradesh, INDIA
2Department of Microbiology, Yogi Vemana University, Kadapa - 516 003, Andhra Pradesh, INDIA
email: jayasree.yvu@gmail.com

INTRODUCTION

Pesticides in soils continue to be studied more than any other environmental contaminant, (Aktar et al., 2009). Organophosphorus compounds, which are a group of highly toxic agricultural chemicals widely used for crop protection, have generated a number of ecological problems such as air pollution, water pollution and dislocating biogeochemical cycles (Zeinat et al., 2008; Cisar and Snyder, 2000). Chlorpyrifos (O, O-diethyl O-(3, 5, 6-trichloro-2-pyridyl) phosphorothioate) is an organophosphorus compound to control insects and mites on a number of crops (Rackeet et al., 1994). Chlorpyrifos is having high soil absorption co-efficient, but low water solubility (Cycon et al., 2009). Environmental Protection Agency (EPA) also reported that water and terrestrial ecosystems are contaminated with chlorpyrifos (Singh and Seth, 1989). Pesticides in soil environment are degraded chemically through reactions such as photolysis, hydrolysis, oxidation and reduction (Bavconet et al., 2003; Kodaka et al., 2003). But chemical degradation is not preferable because of time consumption. A reliable method to detoxify contaminant which is cost-effective for pesticide removal is the biodegradation by bacteria (Serdar and Gibson, 1985). The extent of degradation ranges from formation of metabolites to decomposition to inorganic products (Sassman, 2004; Kaleet et al., 2004). In general, microorganisms demonstrate considerable capacity to metabolize many pesticides (Hindumathy and Gayathri, 2013) they possess the unique ability to completely mineralize many aliphatic, aromatic, and heterocyclic compounds (Bhagobaty, 2007). Many microorganisms can specifically hydrolyze the phosphoester bonds of organophosphates and thus reduce the toxicity of organophosphate pesticides (al-Mihanna et al., 1998; Xu et al., 2008). Considering that chlorpyrifos is one of the most commonly applied insecticides for control of pests and insects, the present experiment was designed to isolate and characterize chlorpyrifos degrading bacteria, to investigate their degradation potential of Chlorpyrifos and to determine their optimum pH and temperature for better biodegradation of contaminated sources.

MATERIALS AND METHODS

Collection of soil samples

soil samples from 10 different locations of the Nandimandalam villagewere collected at YSR district Kadapa in a regular irrigated groundnut fieldswhere chlorpyrifos was applied to control various insect pests. The collected samples were ground to a sieve mesh size of 2 mm and stored at 4°C

Isolation ofchlorpyrifosDegrading Bacteria

Serial dilutionagar plating method is used for the isolation and enumeration of
Characterization of elite Bacteria

The bacteria was purified by adopting standard microbiological procedures and characterized according to the Bergey’s Manual of Determinative Bacteriology (Holt et al., 1994). Bacterial isolates were identified using basic bacteriological methods namely colony morphology, Gram staining and Bio-chemical tests. Isolated bacteria were grown on nutrient broth at 37ºC for 24 h. The Bacterial spent medium was centrifuged at 2000 rpm for 10 min and pellet was used for DNA extraction, the genomic DNA was extracted as described previously CTAB (Cetyl Trimethyl Ammonium Bromide) DNA extraction and purification protocol (Murray and Thompson, 1980). The 16S rDNA gene was amplified by PCR using the universal primers 27Fs'/16F, forward primer at 1492Rs'/16R, (GGYTACCTTGTTACGATT) as reverse primer at 1397. Sequencing was carried out with an automated sequencer (Genetic analyzer 31030, Applied Bio systems). 16S rDNA sequences were compared to other 16S rDNA sequences available in the National Center for Biotechnology Information (NCBI) public database by (BLAST) searching tool. Selected sequences from the database with the greatest sequence similarity to isolated bacterial sequence were aligned and compared. 

Screening for chlorpyrifos degrading bacteria

Bacterial isolates of 24h cultures were used as bacterial inoculum (2300 cells/mL). They were prepared in saline solution (0.85% sodium chloride). 10 μL of bacterial cultures were inoculated into 50 mL of saline and incubated at 37ºC for 3 h. To the respective saline, 2mL of the bacterial inoculum was inoculated into 100 mL Mineral salt broth containing 25, 50, 75 and 100 mg of Chlorpyrifos pesticide and incubated at 37ºC for 24 h in microbial shaker at 150 rpm. Subsequently, 5mL of culture was drawn for 2nd, 4th, 6th, 8th, 10th, and 12th and 14th day. Further 2mL of sample from each concentration was centrifuged at 2000 rpm for 10 min. The supernatant was used to evaluate the growth of pesticide degrading bacteria. The optical density was taken at 560 nm using UV-Spectrophotometer (Kavikarunya and Reetha, 2012).

Effect of temperature on chlorpyrifos degrading bacteria

To study the stability of the bacterial isolates 100 mL of Mineral salt broth was taken into Erlenmeyer flasks sterilized by autoclaving, aseptically transferred 25, 50, 75 and 100mg of pesticides to the flasks and inoculated with 10μL of bacterial cultures to maintained at different temperatures (25ºC, 30ºC, 35ºC and 40ºC). Subsequently, 5mL of culture was drawn for 2nd, 4th, 6th, 8th, 10th, 12th and 14th days of incubation. Further 2mL of sample from each concentration was centrifuged at 2000rpm for 10 min and the supernatant was collected to evaluate optical densityat 560nm using UV-spectrophotometer (Kavikarunya and Reetha, 2012).

Effect of pH on chlorpyrifos degrading bacteria

To study the stability of the bacterial isolates 100 ml of Mineral salt broth was taken into Erlenmeyer flasks sterilized by autoclaving, aseptically transferred 25, 50, 75 and 100mg of pesticides to the flasks and inoculated with 10μL of bacterial cultures to maintained at different pHs (5, 6, 7 and 8). Subsequently, 5mL of culture was drawn for 2nd, 4th, 6th, 8th, 10th, 12th and 14th days of incubation. Further 2mL of sample from each concentration was centrifuged at 2000rpm for 10 min and the supernatant was collected to evaluate optical densityat 560nm using UV-spectrophotometer (Kavikarunya and Reetha, 2012)
RESULTS AND DISCUSSION

Isolation and characterization of chlorpyrifos degrading Bacteria

The isolated bacterium was identified as Gram positive, short rod, catalase positive, oxidase positive and non motile. Primary screening samples were isolated that were capable of utilizing chlorpyrifos at different concentrations (25, 50, 75, 100 mg/L) as the sole source of carbon, the 50 mg/L concentration of chlorpyrifos is optimal concentration. Based on morphological properties of the bacterial colonies, 5 isolates were selected and repeated sub-culture on chlorpyrifos agar (containing 50 mg/L) was performed until uniform colonies were found. Finally by microscopic observation, bacterial isolate was selected, these isolates were investigated further by 16S rDNA studies.

Molecular characterization of elite Bacteria

Isolated bacteria were identified based on their 16S rDNA sequence and BLAST analysis. The results of BLAST analysis of bacterial strains reveal that these strains have the greatest similarity to Bacillus aerius. Similar results has been shown by greatest similarity to Pseudomonas aeruginosa AF137358, P. aeruginosa AF531099, P. aeruginosa AF531099, Pseudomonas aeruginosa EF107515 and Pseudomonas putida AF291048, respectively (Mohammad et al., 2012).

Optimum conditions of chlorpyrifos pesticide degrading bacteria

The relationship of concentration of chlorpyrifos to growth rate of Bacillus aerius was determined by using different concentration methods. Values of specific growth rate (\(\mu\)) obtained from turbidity measurements ranged from 0.1800 to 0.7172. Furthermore, the highest growth rate to 0.7172 occurred at 50mg chlorpyrifos. Above this level, growth was strongly inhibited at 75mg and 100mg (Fig. 3), the estimate of half-saturation growth constants (\(C_s\)) was 33.5 mg chlorpyrifos and the maximum growth rate (\(\mu_{max}\)) was 14th day. Among the four different concentrations, 25 and 50 mg pesticides concentration worked effectively resisted by Bacillus aerius.

Effect of pH and temperature on Chlorpyrifos pesticide degrading bacteria

One of the important abiotic factors that affect the microbial ability towards biodegradation is pH. In connection to this, the growth of pesticide degrading isolates was assessed in mineral salt broth containing 50mg of pesticides at different pH levels viz., 5, 6, 7 and 8 (Fig. 5). The maximum growth rate of bacteria was recorded at pH 6 on 10th day followed by pH 5 at 30°C (Fig-4). The least growth rate was recorded at Ph 7 and 8. Among the four different pH ranges pH 6 was observed as the effective pH for the maximum growth in Bacillus aerius. The growth of pesticide degrading isolates was assessed in mineral salt broth containing 50mg of pesticides at different temperature levels viz., 25°C, 30°C, 35°C and 40°C at pH 6 (Fig. 3). The maximum growth rate of bacteria was recorded at 30°C followed by 35°C and 40°C (Fig-4). The least growth rate was recorded at 45 and 50°C. Among the four different temperature ranges 30°C was observed as the effective temperature for the maximum growth on Bacillus aerius with maximum pesticide degrading capability. The Bacillus spp. shows promising for pesticide removal as they have aerobic and anaerobic mode of lifestyle.

Similarly, (Murugesan et al., 2010) studied the ability of five bacterial isolates (Pseudomonas aeruginosa, Klebsiella sp., Escherichia coli, Bacillus sp. and Corynebacterium) to degrade cypermethrin. It was confirmed that these isolated organisms were able to utilize and degrade cypermethrin. In another research (Kavikarna and Reetha, 2012) represented that, the growth of the three pesticide degrading isolates viz., Pseudomonas fluorescens, Bacillus subtilis and Klebsiella sp. was assessed in Mineral salt broth containing 50mg of pesticides at different temperature levels viz., 25°C, 30°C, 45°C and 55°C. The maximum growth rate of bacteria was recorded at 35°C followed by 25°C and 45°C. The least growth rate was recorded at 55°C.

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REFERENCES


