Identification and Characterization of pesticide degrading *Bacillus cereus* by 16S rRNA Gene Sequencing

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Abstract:

Elimination of pollutants from contaminated sites using microorganisms is a cheaper alternative to chemical technology. There is therefore a need to isolate, identify and characterize the microorganisms that exist and interact in contaminated environment and to isolate the genetic determinants of resistance, frequently located on plasmids. The bacterium was isolated from chlorpyrifos polluted soil. The samples were serially diluted, and the aliquots were incubated for a suitable time following which the suspected colony was subjected to 16S rRNA gene sequencing. The sequence thus obtained was aligned pairwise against *Bacillus* species, which resulted in identification of novel species of *Bacillus cereus.* From this result that the isolated bacteria could be used for the removal of residues of chlorpyrifos in contaminated area.

Key words: chlorpyrifos, microorganisms, *Bacillus cereus,* 16S rRNA.

Introduction

The economy of India is to a great extent rely upon the agricultural production, because of the execution of trend setting innovations as bio fertilizer, chemical fertilizer and diverse types of pesticides have made conceivable to build the quality and amount of field items (Ramudu *et al.,* 2011). Pesticides are the synthetic organic compounds that kill pests like fungi, insects, worms, and nematodes. Which cause damage to field crops. These pesticides have the possible to adversely affect the ecosystem. The excessive use of pesticides leads to accumulation of a huge amount of pesticide residues in the food chain and drinking water environment which may have lethal effects on various living forms (Nayarisseri *et al.,* 2015). The highest quantities of pesticides are accumulated in soil, which may cause changes in the terrestrial environment, often manifested by decreasing soil fertility (Baćmaga *et al.,* 2015).

Among the pesticide, the chlorpyrifos [O,O-diethyl O-(3,5,6,-trichloro-2-pyridinyl) phosphorothioate] is an organophosphorus insecticide extensively used to control pests on grain, fruit, cotton, nuts, vegetables crops, as well as lawns and ornamental plants. US-EPA (U.S. Environmental

Protection Agency, 2002) has been confined for residential uses and even some agricultural purposes in united states and some European countries, but in an India it was continuously use to control insects in agricultural crops (Eaton *et al.*, 2008). The main objective of the present study was to isolate and identify chlorpyrifos resistant bacteria from chlorpyrifos contaminated soil in order to be used for bioremediation of polluted environments.

3. Material and Methods

Isolation of bacteria

Chlorpyrifos was obtained from authorized agro agencies, Chidamparam, Tamilnadu, India. For this study, soil was collected from Soil samples were taken under plant rhizosphere zone. The nutrient agar medium was used for this study. For which the soil were seriously diluted. One gram of soil was mixed with 9 mL of distilled water and mixed thoroughly. One milliliter from the solution was then mixed with 9 mL of distilled water to make 10^{-4} dilution of this solution and in same pattern dilution made up to 10^{-9} dilution. Viable counts for bacteria were determined using a nutrient agar medium containing (per liter of water) the following components; peptic digest of animal tissue, 5.000 g; beef extract, 1.500 g; sodium chloride, 5.000 g; yeast extract, 1.500 g; agar, 15.000 g and finally the pH were adjusted 7.4 ± 0.2.

Screening and acclimation

Several types of colonies were isolated; all colonies that were appeared on the nutrient agar were examined for their chlorpyrifos utilizing capacity. Further, highly potent bacterial strain was acclimatized for the better degrading bacterium.

Identification of chlorpyrifos utilizing bacteria

The potent chlorpyrifos utilizing soil isolate as identified by the methods of morphological, gram characteristic and biochemical tests. Further, 16S rRNA sequencing was done for identification chlorpyrifos utilizing bacteria. The isolated DNA and 16S rRNA gene fragment was amplified PCR from the genomic DNA using 16S gene primers. Commercially available PCR ready mix, primers such as 27F, 5-AGAGTTTGATCMTGGCTCAG-3 and 1525R, 5-AAGGAGGTGATCC AGCC-3. Were used in this study and they were purchased from Chromous Biotech. Pvt. Ltd., Bangalore, India. PCR was performed using standard procedures according to the manufacture's protocol. Briefly, the PCR ready mix 25 µL (containing Taq RNA polymerase, assay buffer and dNTP - mix) was taken in a 0.5 mL PCR tube and each primer of 2 µL (~200 mg of each) was added. Along with that, 2 µL of template RNA (~100 ng) was added and the final volume adjusted to 50 µL with sterile nuclease free water. An overlay of mineral oil 20 µL was added in the reaction tube to avoid evaporation. Control reactions without RNA template were

also prepared. The amplification of the 16S rRNA regions of the bacterial RNA was performed using MyGeneTM Thermal Cycler - MG96G.

The PCR product to be purified was subjected to electrophoresis on 1.2% agarose gel by the method of (Sambrook and Russell, 2001). RNA was visualised by UV trans-illumination. The RNA was then purified using gel extraction kit (Chromous Biotech Pvt. Ltd. Bangalore, India) according to the manufacturer's specifications.

Multiple sequence alignment

The obtained sequenced data were compiled manually and compared with the available sequenced data from NCBI GenBank. Multiple sequence alignment (MSA) is an extension of pair wise alignment to incorporate more than two sequences at a time (Corpet, 1988). MSA method was used to align all the sequences in a given query set. Multiple alignments are often used for identifying conserved sequence regions across a group of sequences hypothesized to be evolutionarily related. Such alignments are also used to aid in establishing evolutionary relationship by constructing phylogenetic tree. Multiple sequence alignment was done using the programme multalin CLUSTAL 2.0.12 multiple sequence alignment (http:// bioinfo. genotoul.fr/multalin/multialin.htmL).

Result

The characteristic features such as shape, gram stain, motality, catalase, indole and oxidase were done for identification of bacteria. The results are exhibited in table-1.

Si.No	characteristics	properties
1.	Shape	rod
2.	Gram stain	+ve
3.	Motality	+ve
4.	Catalase	+ve
5.	Indole	+ve
6.	Oxidase	-ve

Table-1. Morphological and biochemical test of *Bacillus cereus*

Molecular sequencing (16S rRNA) of *Bacillus cereus*

The isolated genomic RNA was used for amplification by using PCR. The PCR-amplification showed the presence of bacterial isolates in the polluted soil, having tolerance against chlorpyrifos. The bacterial isolates were identified on the basis of 16S rRNA phytogenetic sequence by comprising the isolated sequence of base pair and it was conformed that the bacteria were belonged to *Bacillus* species.

The following primers were used for PCR-amplification (27F,5-AGAGTTTGATCMTGGCTCAG-3 and 1525R, 5-AAGGAGGTGATCCAGCC-3). The base pair sequence of primer was complementary to the base pair sequence of bacteria isolated, presence of 520 base pairs (5'CCC….GTA3'), 441 base pairs (5'GCG….GAC3') and 444 base pair (5'GTA….CAT3') in isolated bacteria, the organisms were identified as *Bacillus cereus*. The present study revealed that the isolated bacterial species as identified as *Bacillus cereus*. The result proposed this bacterium isolate may be useful in pesticide contaminated soil for the selective bioremediation of such insecticide.

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