ISSN 2348-1218 (print)
International Journal of Interdisciplinary Research and Innovations ISSN 2348-1226 (online)
Vol. 3, Issue 3, pp: (13-16), Month: July - September 2015, Available at: www.researchpublish.com

Isolation and Characterization of Organo-phosphorous Degrading Bactreium Isolated from Agricultural Soil

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Abstract: **Organophosphorous pesticides are the world's most widely used pesticides in agriculture. Exposure of these pesticides and its metabolities have been related to a variety of nerve disorders in humans. Microbial degradation is considered to be a cost effective method for decontamination of toxic organophosphorous pesticides from the environment. In this study we isolated the bacteria from different agricultural field soil to degrade the Organophosphorous pesticides (Monocrotophos, Dichlorovos, chloropyrifos, diozinon) as a soul carbon source. And the results of biochemical tests shows that bacillus sp. Proteus sp. Serratia sp.effectively degrade the organophosphorus pesticides. 16s rRNA Gene sequencing results show that bacillus sp is responsible for degrading MCP Pesticide than others. Ussing thin layer chromatography technique find out the RF values that confirms the genus. It is evident from the results that the isolated bacillus sp could be used for bioremediate the area contaminated with organophosphorous pesticides. Thus we can remediate the environmental pollution.**

Keywords: **Organophosphorous Degrading Bactreium Isolated From Agricultural Soil, Isolation and Characterization, metabolities.**

1. INTRODUCTION

1.1 Microbial degradation:

Million tons of pesticides were released annually into the environment to protect the crops from damage caused by insects, fungi, weeds, and so on. The term biodegradation is "Breakdown of a substance catalyzed by enzymes". Microbial degradation has advantages because a large variety of compounds can be degraded completely under mild conditions compared with degradation using physical and chemical means.

1.2 Organophosphorous pesticides (OP):

This organophosphorous pesticide is one of the major chemicals responsible for the contamination and deterioration of soil and groundwater, such as monocrotophos, chloropyrifos, dichlorvos (DCV), diozinon.

2. MATERIALS AND METHODS

2.1 Soil Sampling and Culturing:

Soil samples were collected from different agricultural fields such as paddy, sugarcane, gram, grapes and plantain and the data about the pesticides administration was collected.

1g of soil sample was taken and serially diluted. LB medium was prepared. 5µl, 10µl, 20µl, 40µl, 60µl, 80µl, 100µl of pesticide was added in LB medium and agar plate. Finally the broth was inoculated with different dilutions. The medium was incubated at 37ºC for 24 hours.

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2.2 Biochemical Tests:

Morphology test like gram staining(morphology test),motility test, acid fast staining, casein hydrolysis, catalase test, gelatin hydrolysis, indole test, starch hydrolysis, TSI agar test, citrate test, voges proskeur test, oxidase test, urease test, carbohydrate fermentation test, methyl red test, was performed to identify the structure of the organism. Gram staining method was performed to determine the morphology of the organism.

Growth curve:

Growth curve experiment is performed by using various pesticides (MCP, DCV, CHLOROPYRIFOS, DIOZINON) with various concentration to determine the growth of the organism and degradation of pesticides. Optical density (OD) value is taken by using spectrophotometer.

3. THIN LAYER CHROMATOGRAPHY (TLC)

Preparation of TLC plates:

Silica gel was uniformly spread on 15cmx15cm clean glass plates. This silica gel was known as stationary phase. These plate was air dried for few minutes. One line was made about 1 cm on both ends of a glass plate. Samples were spotted on the bare line on the chromatogram plate. Then the plate was placed vertically in a glass chamber containing a solvent is called mobile phase. The mobile phase containing glacial acetic acid and benzyl alcohol in the ratio of 2:1. The sample was moving via capillary action. After 3/4th running of solute that plate was taken out and dried. Finally spray ninhydrin on the plate. After that the band was observed.

Gene Sequencing:

Gene sequencing is a process in which the individual base nucleotides in an organism's DNA are identified. Gene sequencing was done by using sanger method. The sequencing method was done by database of TL/16S_ ribosomal_RNA_bacteria_and _A. Sequencing was done by BLAST analysis.

4. RESULTS AND DISCUSSION

4.1 Biochemical Test Results:

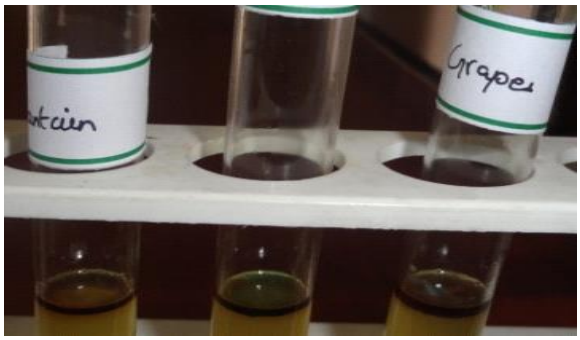

Figure 3: Indole Test

Observation: A red colour ring was observed in the test tubes of plantain, Gram, and Grapes sample.(positive)

Figure 4: TSI agar test

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Observation: H2S production was observed in all samples (rice, plantain, grapes, sugarcane, gram).(positive).

CHROMATOGRAPHY:

Formula:

$$\text{RF Value} = \frac{\text{Distance traveled by solute}}{\text{Distance traveled by solvent}}$$

Table 1:

SAMPLE	RF VALUE
Sample 1	**0.75**
Sample 2	**0.75**
Sample 3	**0.77**
Sample 4	**0.78**
Sample 5	**0.67**

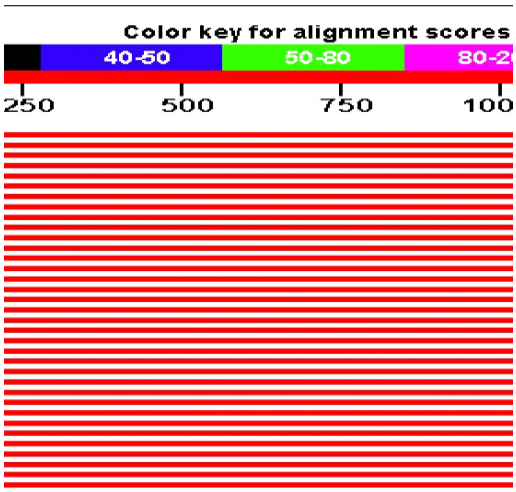
Thin layer chromatography analysis was performed for all the samples such as (rice, sugarcane, plantain, gram, grapes). Different Rf values were obtained from the analysis. From the above Rf values we discussed that these Rf values confirms the organisms are Proteus sp., Bacillus sp.

TLC method was performed for monitoring the cell growth and dissipation of chloropyrifos. The method was carried out for the characterization of organism (Harish. R *et al*., 2012).

4.4 Degradation Profile:

In this study we monitored the degradation of pesticides using LB medium. The particular colony of bacillus clausii KSM-K16 was isolated and growth curve experiment was performed. These experiment was performed with different doses of organophosphorous pesticides in order to determine the optimum concentration of OP pesticides that stimulates the growth of Bacillus clausii KSM-K16.

Basic Local Alignment Search Tool:



5. CONCLUSION

Degradation Profile:

After 60 hours of incubation the degradation of pesticides were analysed using spectrophotometer. The various concentration of pesticides includes 20µl, 40µl, 60µl. Microbial enrichment culture composed of Bacillus sps., Proteus sps., were found to degrade diazinon in more amount compare to other pesticides. Present findings reveal the order of degradation of the four pesticides was as follows; diazinon> chlorpyrifos>monocrotophos>dichlorvos.

As our biochemical test results and gene sequencing report shows bacillus sp more effectively degrade the pesticides.

Gene sequencing confirms the organism was bacillus sp such as

- bacillus clausii KSM-K16 strain
- bacillus clausii DSM 8716 strain
- bacillus rhizosphaerae SC-N012 strain
- bacillus lehensis MLB22 16S strain
- bacillus xiaoxiensis JSM 081004 16S strain

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