Research Article

EVALUATION OF MONOCROTOPHOS DEGRADATION BY Lysinibacillus sphaericus JX012235 ISOLATED FROM SWINE WASTE

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Abstract- In India, the mainly used pesticide in agricultural crops is Monocrotophos, an organophosphorus pesticide. It is denoted as extremely toxic and their exposure to environment is considered to be lethal. In this study, degradation of Monocrotophos by Lysinibacillus sphaericus JX012235 a bacterium isolated from swine waste was studied. Growth studies of the bacteria in mineral salt broth supplemented with the pesticide at the concentration of 100-1000 ppm initially confirmed the degradation of the pesticide. The bacterium was found to survive when pesticide was given as a sole source of nutrition. The pesticide degradation was confirmed by LC-MS. The phosphatase activity of bacterium was found to be 69U/ml.

Keywords- Monocrotophos, Lysinibacillus sphaericus, Biodegradation, Swine waste, Phosphatase activity.

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Introduction

The growing population of the world has given more pressure to the field of agriculture in terms of food, nutrition etc. Hence there has been a huge utilization of technology, new varieties and short term crops, usage of fertilizers, pesticides and insecticides to increase the crop yield. The agricultural land should be supplied with a minimal amount of chemicals so that it does not affect the land’s fertility and the water washed off to rivers. The commonly used pesticides are Monocrotophos, Dichlorovos (Organophosphates), Parathion, Diazinon (Organophosphates), Vanimidion (Thiophosphates), Phorate, Malathion (Diithiophosphates) and Trichloron (Phosphonates). These pesticides have their own permissible levels in land. However, if the concentration exceeds the normal level the fertility of the soil is lost and the pesticide retains there causing environmental pollution. The pesticide infestation in soil can cause severe problems like 1) alterations of ecological balance, 2) loss of common soil microflora 3) Less soil fertility and crop productivity 4) Interference with ammonification in soil 5) alterations in rhizosphere microflora 6) absorption of pesticides by plants. Monocrotophos, a relatively cheap pesticide is principally used in agriculture. Wistar rats in a recent study were administered with 1/50th of LD50 dosage of monocrotophos (0.36 mg/kg body weight) for three weeks orally. Dyslipidemia and mild hyperglycemic conditions was observed in blood when monocrotophos was administered in animals. Cardiac oxidative stress was conferred by accumulation of protein carbonyls, lipid peroxidation and glutathione production [1]. Monocrotophos on contact is highly toxic to organisms, including humans [2]. The Lysinibacillus sp which was isolated from contaminated agricultural soil was found to degrade fomesafen, an herbicide and it was identified using the comparative analysis of 16sRNA gene [3]. However, there are only few reports where Lysinibacillus sphaericus has been used in the pesticide degradation. Swine waste from pig farm is often used as manure in agricultural land. When manure is used on the agricultural land, the impact on the environment has to be considered. If the manure has got the ability to degrade pesticides and heavy metals it will be more useful for farmers. Hence the present study is aimed to isolate and to find out if there is any pesticide degrading bacteria present in the swine waste.

In this study Lysinibacillus sphaericus isolated from swine waste was tested for the efficacy of pesticide degradation. Lysinibacillus sphaericus, a Gram-positive, mesophilic, rod-shaped bacterium is known for its bioremediation capability. It was initially isolated from a mosquito breeding site in China in 1987 [4]. This bacterium was found to be more effective in pesticide degradation. It can surely be used for the direct application in the agricultural field because of its capacity to grow in low nutrition conditions.

Materials and Methods

Isolation and Identification of Bacteria from swine waste

Fresh swine waste was collected from TANUVAS Research Station, Kattupakkam and the filtrate was prepared using 10mL of Sterile Distilled water. The sample was then serially diluted and the bacterial colonies have been isolated from different dilutions ranging from 10-6 to 10-9. The bacterium was then streaked repeatedly in nutrient agar to obtain pure cultures. The isolated strain was then identified by biochemical tests using himedia Biochemical test kit (KB001) and 16s rRNA sequencing using the primers: Forward – 5’ (5’ – GAGTTGTACATGGCTCGAG-3’) Reverse – 1495r (5’-CTAGGCTACCTTGTACGC-3’) [5].

Evaluation of Pesticide degradation by Lysinibacillus sphaericus

The Bacteria Isolated from Swine Waste Was tested for degrading Monocrotophos (36% SL). The bacterium was initially inoculated both in Mineral salt agar devoid of carbon source (MgSO4 – 0.05g, Fe(SO4)3 – 0.01g, NaN03 – 2.0g, KH2PO4 – 0.14g, K2HPO4 – 1.2g, KCl – 0.5g, Yeast extract – 0.02g, Agar – 15g, pH - 7.2) and Mineral salt medium (MSM) supplemented with pesticide in the range of 100-1000 ppm initially confirmed the degradation of the pesticide. The bacterium was found to survive when pesticide was given as a sole source of nutrition. The pesticide degradation was confirmed by LC-MS. The phosphatase activity of bacterium was found to be 69U/ml.
1000ppm respectively under aseptic conditions. Uninoculated media with the pesticide served as a control. The MSM containing test tubes were incubated under shake culture condition (100rpm) at 37°C for 6 days. Each day the absorbance was checked in UV spectrophotometer at 600nm. The growth curve was plotted accordingly to know the actual growth of the bacterium.

**Medium optimisation with Monochrotophos**

The bacterium was then inoculated in modified Mineral salt medium supplemented with 1000ppm of Monochrotophos i.e MSM without MgSO\(_4\), MSM without FeSO\(_4\), MSM without KCl, MSM without KH\(_2\)PO\(_4\), MSM without KH\(_3\)PO\(_4\) and MSM without NaNO\(_2\). In all these media, the yeast extract was neglected to remove the nitrogen source completely. The bacterium was inoculated in the above prepared sterilized media individually and their growth was checked in UV-Spectrophotometer at an absorbance of 600nm. In this experiment, the bacteria was inoculated in 2% pesticide agar containing maximum amount of Monochrotophos -1000ppm and 2000ppm and tested whether it could be able to grow only in the presence of pesticide without any additional nutrients. The growth was monitored continuously for 7 days [6 & 7].

**LC-MS analysis of Monochrotophos degradation**

After the optimization of Monochrotophos degradation by testing them in various media containing nutrients as well as MCP of about 1000ppm, the sample was collected at the end of 7\(^{th}\) day and centrifuged at 8000rpm for 25 minutes. The cell-free supernatant was estimated for residual content of Monochrotophos by LC-MS (Model –LCMS 2020 Shimadzu) using UV detector. The column used was C\(_{18}\) reverse phase Column with size 250x4mm.5 micron size. The operating conditions were as follows: column temperature - 32°C; mobile phase, 20% acetonitrile in water; flow rate 0.2ml min\(^{-1}\); volume of sample injected , 3µl. Standard aqueous solution of Monochrotophos of concentration 1000ppm was tested in LC-MS which served as a control [8].

**Determination of Phosphatase activity**

Alkaline phosphatase activity was measured by the release of p-nitrophenol from p-nitrophosphoryl compound (pNPP) at 400nm [9]. A typical reaction mixture contained 100 µl of enzyme diluted in 2ml of Tris buffer (pH 8.5), 100 µl of 5mM CaCl\(_2\), 100 µl of 500 µmol pNPP and the final volume was made up to 3ml using distilled water. Blank was prepared by mixing 2ml of 200mM Tris buffer (pH 8.5) and 1ml distilled water. The reaction was performed at 37°C for 30min and stopped by addition of 500µl of 4M sodium hydroxide. One unit of phosphatase is the amount, which hydrolyses 1 µmol of substrate per min. The standard curve obtained by absorbance of p-nitrophenol (0 - 500 µmol) at 400nm was used for quantification of enzyme activity [10].

**Results**

**Isolation and Identification of pesticide degrading bacteria:**

Totally five isolates designated as D1, D2, D3, D4 and D5 obtained from the swine waste have been checked for the pesticide degradation. Among this D2 had the maximum capacity to grow in Minimal salt agar supplemented with the pesticide. The culture D2 was identified as Lysinibacillus sphaericus by 16s RNA sequencing. The obtained sequence was deposited in the Genbank and the strain and accession number obtained are DMS 3 and JX012235. The phylogenetic tree for the bacteria and the photomicrograph is given in [Fig 1a] and 1b) respectively.

**Evaluation of Pesticide degradation by Lysinibacillus sphaericus**

The growth curve was plotted [Fig-2]. It was found that the growth was very prominent in media containing MCP of concentration 100ppm and minimum growth was observed at the maximum concentration of 1000ppm.

**Discussion**

This is the first report of Lysinibacillus sphaericus being used for the pesticide degradation especially Monochrotophos, an organ phosphorus compound. Lysinibacillus sp was also reported for fomesafen, an herbicide degradation which

![Fig-2](image-url)
was isolated from contaminated agricultural soil. The strain was reported to utilise fomesafen as the sole carbon source for growth and the total degradation rate was 81.32\% after 7 days of incubation [3].

Monocrotophos degradation by Paracoccus sp M-1 isolated from sludge of a wastewater treatment system was reported by Jia et al. They reported that Paracoccus sp M-1 could use MCP as a sole carbon source and also reported 79.92\% degradation of MCP after 6hrs of incubation. The degradation of the pesticide supported cell growth indicating that M-1 could utilise it [11]. In the present study Lysinibacillus sphaericus could also use MCP as sole source of carbon without addition of any nutrients. MCP degrading Aspergillus sp. and Pseudomonas aeruginosa was isolated from the soil contaminated with monocrotophos by Athul Sanjeev and Roopa [12].

The degradation of MCP by Lysinibacillus sphaericus has the capacity to grow in adverse conditions and they form spores during the unfavourable conditions. In the present study Lysinibacillus sphaericus isolated from swine waste could possibly be used in pesticide contaminated soil for Monocrotophos degradation.

**Conclusion**

Lysinibacillus sphaericus isolated from swine waste have been found to degrade monochrotophos and can be used in future for Biodegradation of monochrotophos in pesticide polluted soil.

**Application of Research:** This study could be useful for biodegradation of Monochrotophos polluted soil.

**Research Category:** Bioremediation

**Abbreviations:** ppm- parts per million, LD- lethal dose, LC-MS – Liquid chromatography Mass spectrometry, MSM- mineral salt medium, MCP - Monochrotophos

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**Conflict of Interest:** None declared

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