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TRANSFORMATION OF *RHIZOBIUM* WITH PLASMID FROM *PSEUDOMONAS SPP.* A 1113 TO DEGRADE DIMETHOATE

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ABSTRACT

Dimethoate is a group of organophosphorus pesticide which inhibits the enzyme cholinesterase and hence causes neurological disorders in insects, humans and pest. Rhizobium forms an endosymbiotic nitrogen fixing association with roots of leguminous plant. Present study includes transformation of Rhizobium which is a genus of gram negative soil bacteria that fix nitrogen. Symbiotic nitrogen fixation is generally the dominant source of nitrogen input in soil for imparting fertility but also avoid soil stresses, such as temperature, acidity and salinity which pose a severe yield constraint in obtaining plant growth and development. In this study, Rhizobium was isolated from leguminous plant which did not show growth on the medium containing pesticide as a sole source of carbon. This Rhizobium was used for transformation with plasmid isolated from Pseudomonas spp. A1113 to degrade dimethoate. Rhizobia were successfully transformed with Pseudomonas spp. A1113 as growth was observed on the YEM medium containing Dimethoate.

KEY WORDS

Dimethoate, Rhizobium, Plasmid, Transformation.

INTRODUCTION:

Pesticide is a substance or mixture of substances intended for preventing destroying or controlling any pest including vectors of human or animal disease, unwanted species of plants and animals causing harm during or otherwise interfering with the production, processing, storage, transport or marketing of food, agricultural commodities, wood and wood products or animal foodstuff, or substances which may be administered to animals for the control of insects, arachnids or other pests in or on their bodies. The term includes substances intended for use as plant growth regulator, defoliant, desiccant or agent for thinning fruit or preventing the premature fall of fruit. Also used as substances applied to crops either before or after harvest to protect the commodity from deterioration during storage and transport. [3]

Dimethoate is used in agriculture and is an ester or thiol derivatives of phosphoric, phosphonic or phosphoramidic acid [5]. Organophosphorus pesticides are highly toxic and easily absorbed through the skin. Poisoning may also occur through the mouth. There are many effects when inhaled. The first effects are usually respiratory and may include bloody or runny nose, coughing, chest disorder, difficult or short breath. These may include vomiting, Diarrhea, abdominal cramps, headache, eye pain and blurred vision. Severe poisoning will affect the central nervous system lack of co-ordination & eventually paralysis of the body extremities & respiratory muscles. [1]

Despite their high toxicity, Dimethoate pesticides are still extensively used all over the world for its broad spectrum of action. Dimethoate inhibits the enzyme choline esterase which is required for normal

functioning and hence causes severe neurological disorder in humans [2]. Present study includes transformation of *Rhizobium* which cannot degrade dimethoate. *Rhizobium* forms an endosymbiotic nitrogen fixing association with roots of leguminous plant which is a genus of gram negative soil bacteria that fix nitrogen. Symbiotic nitrogen fixation is generally the dominant source of nitrogen input in soil for imparting fertility but also avoid soil stresses, such as temperature, acidity and salinity which pose a severe yield constraint in obtaining plant growth and development. In this study, *Rhizobium* isolated from leguminous plant which did not show growth on the YEM medium containing pesticide as a sole source of carbon. This *Rhizobium* was used for transformation with plasmid isolated from *Pseudomonas spp.* A1113 to degrade dimethoate. *Rhizobia* were successfully transformed with *Pseudomonas spp.* A1113 as growth was observed on the YEM medium containing Dimethoate. Hence, this transformed *Rhizobium* can be used for both pesticide degradation and nitrogen fixation which will promote plant growth.

MATERIAL AND METHODS:

1. **Isolation of *Rhizobium* from leguminous plant:**
   Fenugreek (Methi) plant nodules were collected from the roots, washed with sterile water followed by surface treatment with 95% alcohol and again with sterile water. The nodules were transferred in culture tube half filled with sterile water and crushed with a sterile glass rod to obtain a milky bacterial suspension. Suspension was streaked on Congo Red Yeast Extract Mannitol (CRYEMA) agar plates and incubated for 2-3 days at 28^0C. [3]
2. **Isolation of plasmid:**
   Plasmid isolation using miniprep system was performed on the isolate showing maximum pesticide degradation. [7]
3. **Transformation in *Rhizobium:***
   Rhizobium was inoculated in YEM broth and incubated overnight for 250 rpm at 28^0C.The cells were harvested by centrifugation at 10000 rpm for 10 min at 4^0C; suspended in 10ml ice cold 100mM $CaCl_2$ and incubated in ice for 60 min. The cells are then suspended in 10ml ice cold 100mM $MgCl_2$ and again incubated in ice for 60 min. Then centrifuged at 10000 rpm for 10 min at 4^0C, the pellet was resuspended in 3ml of ice cold 100mM $CaCl_2$ and left overnight. 20 µg of plasmid DNA was taken and added it to 200 µl of competent cell suspension, mixed gently and kept on ice for 30min.Heat shock was given at 42^0C for 2 min immediately transferred to ice bath for 1 hr. To this 1.8 ml of sterile LB broth was added, incubated at 28^0C on shaker for 1 hr for the cells to recover.100 µl of cell suspension was inoculated on YEMA plates with dimethoate 0.1% and incubated for 24hrs. [7]

RESULT AND DISCUSSION:

Rhizobium was isolated from roots of fenugreek plant. Pink color colonies on CREYMA were selected for transformation. [9] No growth observed on Hofer's Alkaline medium confirms that the isolate belongs to *Rhizobium* genus.

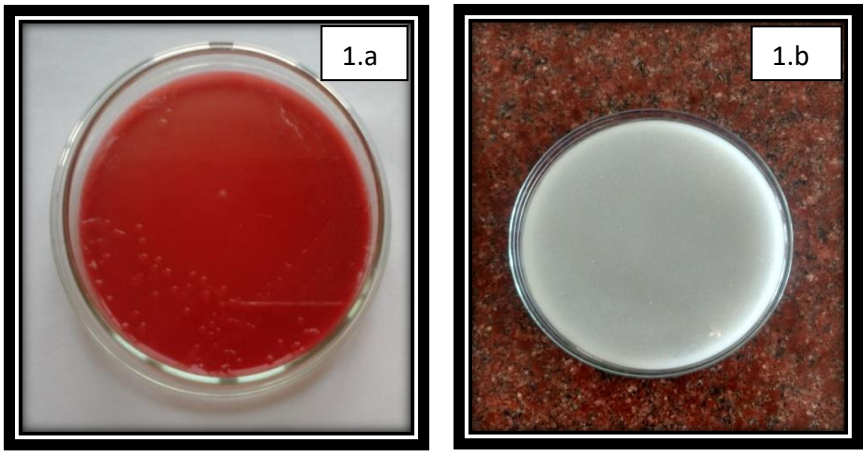

Figure 1:
a) Pink color colonies on CREYMA indicated *Rhizobium* b) No growth observed on Hoffer's alkaline medium confirms *Rhizobium* genus.

As discussed by Barbara E. Kneen Congo red absorption is generally considered a contraindication of *Rhizobium*. The uptake of congo red varies among strains of *Rhizobium*, as shown elsewhere with strains of R. tiriitolii and R. meliloti. Congo red absorption does not distinguish rhizobia from other bacteria but may be useful as a strain marker. Hofers Alkaline Medium is formulated as described by Subba Rao and is used for growing Agrobacterium species while inhibiting Rhizobium species from soil. It is a selective medium with high alkaline pH. Agrobacteria grow at higher pH while Rhizobia fail to grow at alkaline pH. [8]

In previous studies, at secondary screening *Pseudomonas spp.* A1113 was isolated which degrades pesticides and was selected for further studies [12]. The mode of degradation was found to be plasmid mediated. [13]

Figure 2: Plasmid bands observed under U.V trans-illuminator after gel electrophoresis. Lane 1 from right contains PUC18 (Control) and lane 2 shows the plasmid band isolated from *Pseudomonas spp.* A1113

Rhizobium was transformed with plasmid isolated from *Pseudomonas spp.* A1113. To determine whether the dimethoate was plasmid mediated the transformed *Rhizobia* was grown in the YEM medium containing dimethoate (0.1gm%). Transformants were obtained on the medium containing pesticides which show the ability to utilize pesticide as a sole source of carbon. However, these results can be confirmed after plasmid curing experiments. [10]

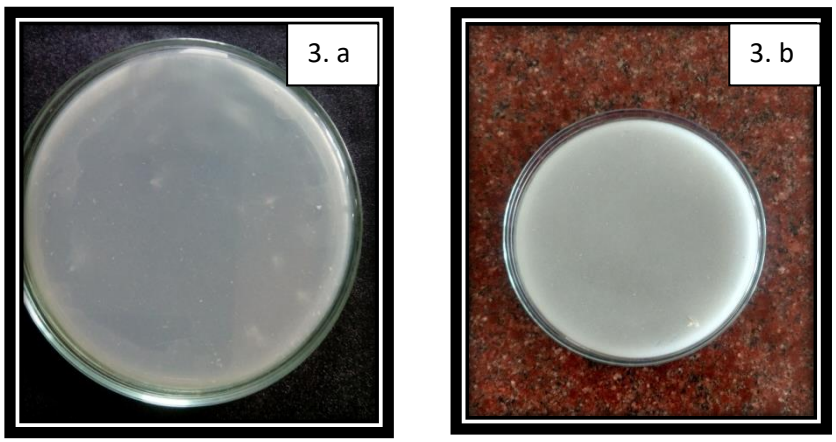

Figure 3: a. Transformants having ability to grow on YEM containing Dimethoate (0.1 gm %). b. Non Transformants show no growth on YEM containing Dimethoate (0.1 gm %).

CONCLUSION:

In this study, biological degradation of dimethoate has been carried out using soil isolates which include bacterial isolates. Bacterial isolate identified as *Pseudomonas spp.* A1113 can biologically degrade this pesticide. Isolation of *Rhizobium* from Leguminous plant was carried out. The Dimethoate degrading property of organisms was plasmid mediated which was confirmed by isolation of plasmid DNA. [13] Isolated plasmid from *Pseudomonas spp.* A1113 was transformed into Rhizobium and transformants were obtained on the media containing can be used in bioremediation as it possesses dual properties i.e. nitrogen fixation as well as pesticide degradation.

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