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Evaluation of Malathion Degradation by Bacterial Isolates from Agricultural Soil

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A B S T R A C T

Chemicals like pesticides, which are heavily used in Indian agriculture, have a negative influence on the environment, biodiversity, and the health of people and animals. The aim of this investigation is to find soil microbes that can break down the pesticide's malathion. This investigation shown *Serratia marcescens* and *Pseudomonas aeruginosa* as the two bacterial isolates that demonstrated a significant capacity to carry out the breakdown of malathion.

Introduction

Asia utilizes more than half of the world's pesticides. In terms of pesticide use, India ranks 12th globally and third in Asia. Multiple studies have revealed that pesticides are present in India's food, environment, and biological systems, including blood samples and breast milk.

Pesticide residues were detected in 18.7% of samples, unapproved pesticides were found in 12.5% of samples, and residues above the maximum residue level (MRL) recommended by FSSAI were found in 2.6% of samples, according to a recent report published by the All India Network Project on Pesticide Residues (Bhatt *et al.*, 2021). Pesticides for agriculture, public health, and household use are registered in India. As of October 30, 2016, there were 275 pesticides registered for use in India, of which approximately 255 are chemical poisons (Dileep Kumar, 2017).

Malathion is a prominent organophosphate insecticide used to control a wide range of pests, including mosquitoes, fruit flies, and aphids. It inhibits the activity of the enzyme acetylcholinesterase, which is necessary for the normal functioning of the nervous system. Malathion is therefore exceedingly toxic to insects and other organisms with this enzyme (Singh *et al.*, 2014).

Malathion is typically applied as a liquid spray or a dust to crops, gardens, and other outdoor areas for insect control. Malathion is an effective pesticide, but it is also toxic to beneficial insects and fauna, and it can persist in the environment for an extended period of time (Ullah *et al.*, 2018).

Malathion is categorized as a probable human carcinogen by the International Agency for Research on Cancer (IARC) and can cause a variety of adverse health effects in humans, such as migraines, dizziness, and nausea. Long-term exposure to malathion can also increase the risk of neurological side effects like tremors and coordination difficulties (Asim *et al.,* 2021).

Pesticide degradation typically involves a combination of processes, such as microbial degradation and chemical hydrolysis, and is influenced by physicochemical properties such as temperature, pH, and carbon and nitrogen sources (Kumar, 2013). However, biodegradation is the primary pesticide degradation and detoxification mechanism in soils. Therefore, bacteria and other microorganisms may have a significant impact on the persistence of the majority of organophosphate pesticides in soil (Mansouri *et al.,* 2017). Biodegradation is a prevalent method for the removal (degradation and detoxification) of organophosphate pesticides due to its low cost and low collateral damage to native animal and plant organisms (Maurya *et al.,* 2016). The bacterial degradation of Malathion and other organophosphorus pesticides is a significant factor in determining their fate in the environment.

The ability of various bacterial strains found in agricultural soil to degrade malathion has been investigated in an in vitro biodegradation investigation. Following screening, two isolates were chosen and identified, injected in a pure liquid culture medium that had been supplemented with a known quantity of malathion, and then incubated. Samples were taken in tandem with the control sample at regular intervals.

Materials and Methods

Soil samples collection and preparation

In the district of Gwalior, Madhya Pradesh, soil samples were collected from agricultural areas. 0–15 cm of surface soil were collected, deposited in plastic bags, transported to the laboratory on ice, and stored at 4°C until analysis. Prior to bacterial testing, soil samples were air-dried and passed through a 15-mm-mesh sieve.

Isolation and Screening of Malathion degrading bacteria

Ten grams of soil sample were mixed into 100 ml of MSM and supplemented with 25 ppm Malathion. Samples were cultured on a rotary shaker (150 rpm) at 30°C for 7 days before being transferred to a fresh medium and incubated under the same conditions for another 3–4 days, or until increasing turbidity were seen. Following 2-3 rounds of sub-culturing, 0.1 ml of culture broth was pipetted and disseminated over MSM + Malathion agar.

Single colonies were chosen and streaked onto MSM containing 25, 50, 75, and 100 ppm of the insecticide Malathion. For three days, cultures were incubated at 30°C. Malathion-degrading isolates were chosen among isolates that generated a clear growth around their colonies when grown on MSM supplemented with 100 ppm Malathion.

Biodegradation of Malathion by bacterial isolates

Bacterial isolates which is shown a significant growth on 100ppm concentration were cultivated in MSM + Malathion and incubated for 10 days on a rotary shaker at 150 rpm and 30°C. Bacterial cell growth was measured using samples collected every 24 hours. A spectrophotometer was used to measure the growth in terms of optical density (OD) at 600 nm.

Each sample was vortexed with two ceramic homogenizers to assess pesticide levels. After adding 10 mL of acetonitrile (ACN), the sample was vortexed for 2 minutes. Six milliliters of the extract were transferred to the general QuEChERSdSPE (p/n 5982-5056). The materials were then vortexed for 2 minutes before being centrifuged at 5,000 rpm for 5 minutes. The extract was subjected to GC-MS analysis.

Characterization of bacterial isolates

Morphological studies

Isolates of bacteria were grown in MSM+malathion and incubated at 30^0C until colony formation was observed. Under a microscope (1000x magnification), Gram staining and cell morphology were observed.

Molecular characterization of Isolated Malathion-Degrading Bacteria

The CTAB-phenol-chloroform-isoamyl alcohol extraction procedure was used to obtain genomic DNA for amplification and sequencing of the 16S rRNA gene of effective malathion degrading bacterial isolates (Ausubel *et al.*, 1997). The efficient bacterial isolate's 16S rRNA fragment was amplified using universal primers 16sR (5' CGGTGTGTACAAGGCCCGG 3') and 16sF (5' GGATGAGCCCGCGGCCTA 3'). The following conditions were used for the PCR reaction: 7.30 min at 96 °C, followed by 30 cycles of 3 min at 94 °C, 1 min at 94 °C and 1 min at 50 °C,2 min at 72 °C, 7 min at 72°Cand hold at 4oC.

Purified PCR product was sequenced using an automated DNA sequencer (Applied Biosystems (ABI), 3130 Genetic Analyzer) as the usual Sanger di-deoxy method. Using BLAST (Basic Local Alignment Search Tool) (http://www.ncbi.nlm.nih.gov/blast/Blast), the acquired sequence was examined and compared with the database of the previously sequenced organism. The sequence was submitted to the National Center for Biological Information's (NCBI) Genbank. MEGA X software was used for multiple sequence alignment and the creation of phylogenetic trees (Kumar *et al.,* 2016).

Statistical analysis

Experiments were carried out in triplicates, and the results presented are the mean ± standard error of the three replicates.

Results and Discussion

Isolation and morphological characterization

From 15 different soil samples, a total of 35 isolates were collected. The majority of the isolates were gram-negative cocci or bacilli, according to microscopic examination.

Screening of isolated

After isolation, all the 35 isolates were tested for their resistance to pesticide at 4 concentration viz. 25, 50, 75 and 100ppm in term of growth.

Out of 35 isolates, 15 isolate showed the significant growth on concentration of 100 ppm, were further selected for their study.

Biodegradation of malathion

The spectrophotometric method was used to examine preliminary malathion degradation of selected 15 isolates. After 10 days of incubation in shaking conditions, 89% and 91% degradation were observed for S3MaA and S11MaA for 100 ppm of monocrotophos, respectively (Figure 2).

GCMS was used to perform a confirmatory monocrotophos degradation study. S3MaA and S11MaA had malathion degradation potentials of 91% and 93%, respectively. The standard malathion retention time was 9.362 minutes, while S3MaA and S11MaA were found at retention periods of 9.362 minutes and 9.426 minutes, respectively, confirming the presence of malathion in the medium (Table 1; figure 3 and 4).

Identification of Isolates

The microscopic analysis indicated that isolate S3MaA was found to be rod-shaped Gram-negative, while colonials features showed red pigment production with a smooth colony surface. S11MaA had green diffusible pigmentation, translucent, alveolate surface, and Gram-negative staining.

Table.1 GCMS analysis of Concentration and degradation of Malathion

Isolates	Concentration of Malathion in MSM medium(ng/ml)		Percentage of Malathion Degradation
	Initial concentration (ng/ml)	Final concentrationng/ml)	
S3MaA	20.5621	1.8714	91%
S11MaA	20.5621	1.5117	93%

Fig.1 Selected isolates exhibiting significant growth at 100 ppm Malathion concentration.

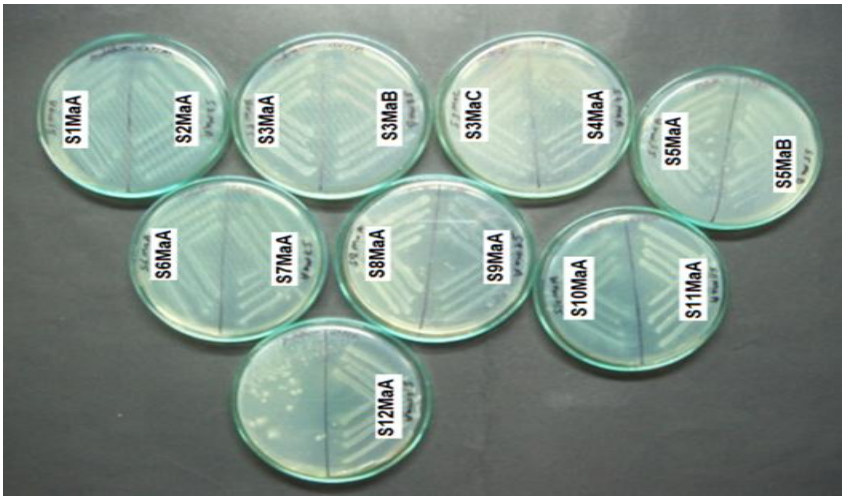


Fig.2 Spectrophotometry investigation of Malathion degradation by selected isolates.

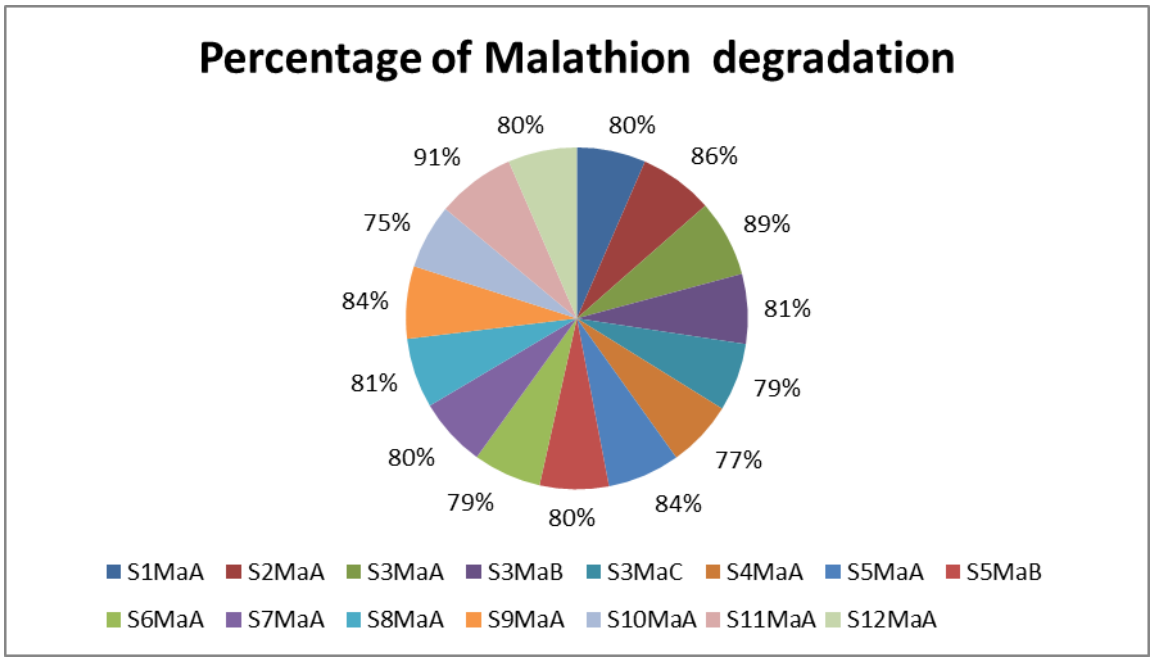


Fig.3 S3MaA isolate quantitative study of Malathion

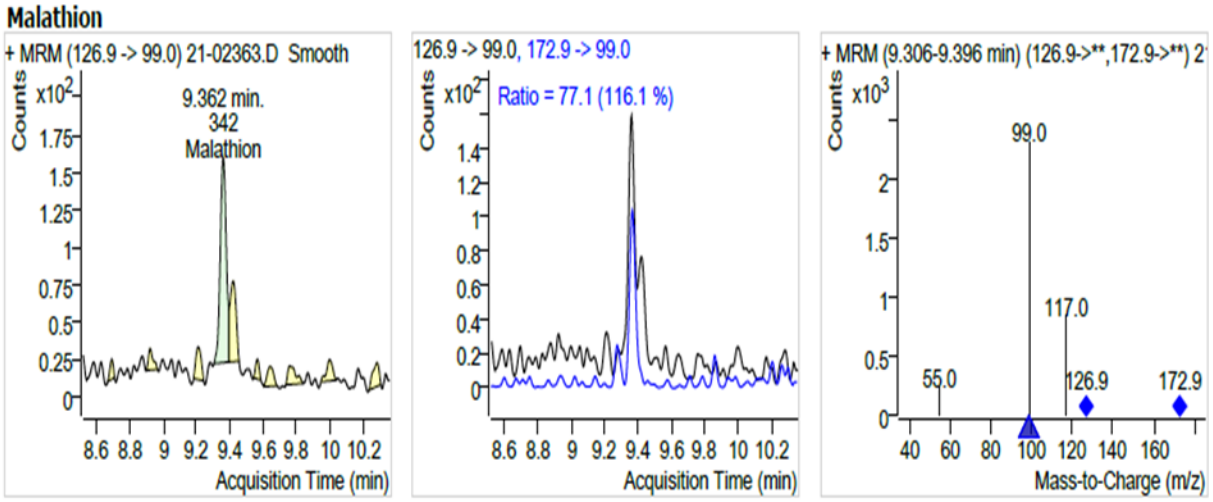


Fig.4 S11MaA isolate quantitative study of Malathion

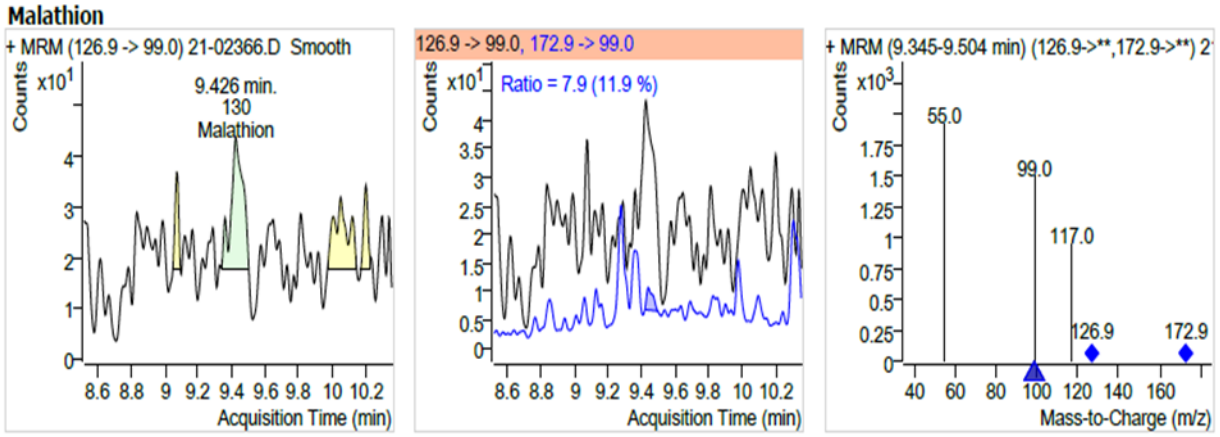


Fig.5 Phylogenetic tree based on 16S rRNA sequence analysis constructed by neighbor joining method by using MEGA X

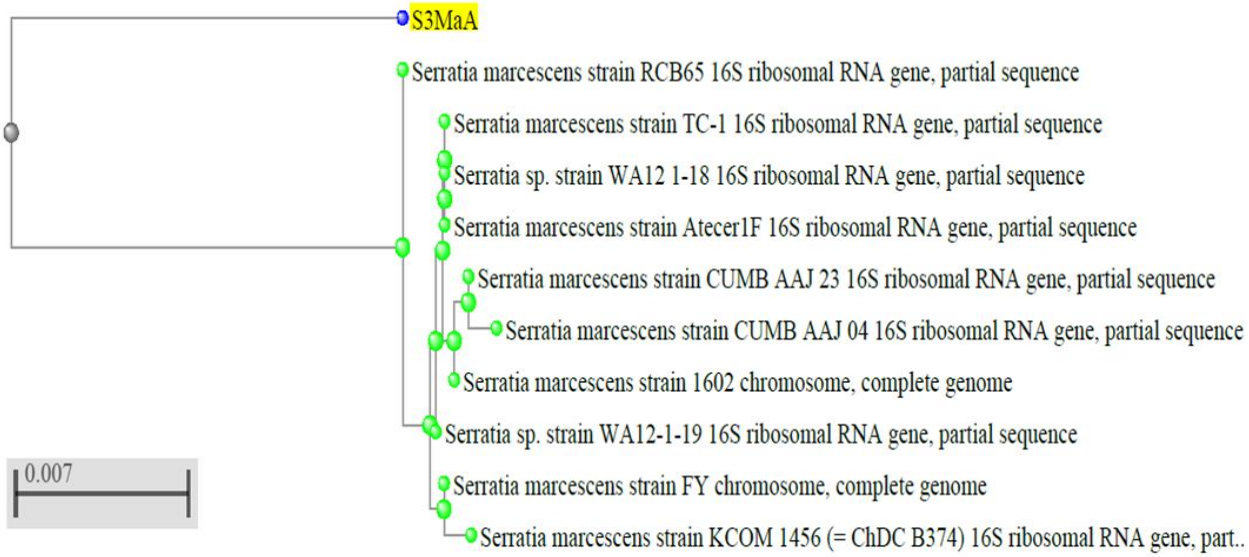
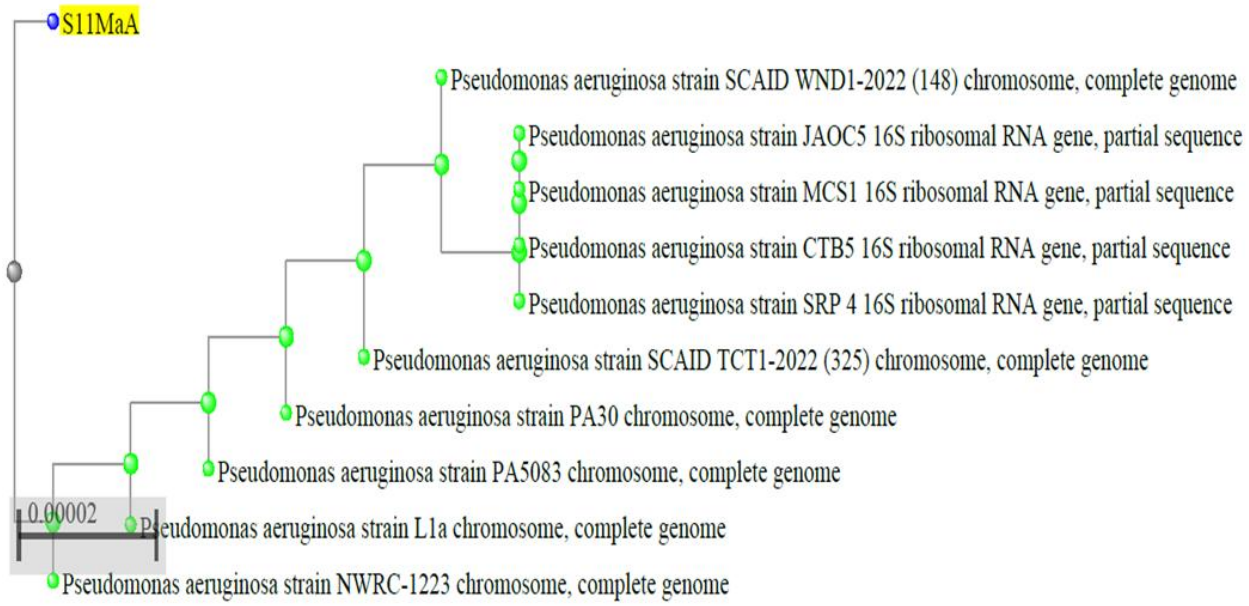


Fig.6 Phylogenetic tree based on 16S rRNA sequence analysis constructed by neighbor joining method by using MEGA X



16s rRNA gene sequencing indicated that the coded S3MaA and S11MaA were identified as *Serratia marcescens* and *Pseudomonas aeruginosa* consequently. The nucleotide sequences were submitted to the NCBI gene bank with accession numbers LC761572, and LC761573**.**

In conclusion, the current study covers the isolation, characterisation, and identification of malathion-degrading bacterial isolates. A large number of indigenous strains of bacteria capable of breaking down malathion could be identified from cultivated areas with a known history of pesticide treatment. These isolated strains of bacteria are well adapted to their surroundings and could thus be used for bioremediation and metabolic detoxification of malathion.

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