Isolation and characterization of potential Malathion tolerating bacteria from pesticides polluted soil

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Abstract

Pesticides play an important role in agriculture in developing countries, especially in India. Their significance in improving crop yield is well known and understood. The Continuous use of pesticides in fields is harmful as some pesticides remain in the soil for longer periods of time and enter the food cha in that affects animals and humans. The current study is aimed at developing the method of biodegradation to reduce the pesticide's environmental burden. In the present study, Malathion degrading bacterial isolates have been isolated from various cultivated soil samples. The different isolates were characterized by various biochemical tests for identification. Among the 22 isolates, a potential isolate of *Bacillus pumilus* was utilized for the optimization process. The degradation level was determinate with an optical density of biomass. Furthermore, the potential isolate was subjected to the production of Organophosphorous phosphatise, after dialysis, 51.04U/ml of the enzyme was observed. Thus, this isolated bacterial species could be used as potential candidates for bacterial mediated bioremediation of pesticide.

Key words: Pesticide degradation**,** Malathion, *Bacillus pumilus*, Organophosphorous phosphatase

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I. INTRODUCTION

Pesticide residue is the definite source of pollution of land and soil. A list of such pesticides in use is quite lengthy. The most important ones like Dichlorodiphenyltrichloroethane (DDT), malathion, para-malathion, adrian, dialdrins, cause a serious problem of land pollution. The characterization and determination are not simple. In worldwide, more than 500 components are registered and used as pesticides. Amongst the South Asian countries, India is the highest pesticides country, and most commonly used pesticides in India include organophosphates, organochlorins, neonicotiniods etc (Satish *et al*., 2017).

Some pesticides have a deleterious effect on health. Several of them are carcinogenic and cause long term harmful effects upon health and hence need urgent analysis. As per the definition of perfect pesticide, a pesticide must be harmful to the targeted pests, but not to non-target species, including man but sadly, this is not so, hence the dispute of use and abuse of pesticides has come into the light. Due to unplanned and indiscriminate use, only ten percentage of applied pesticides capacity the target organism and the remaining more percentage of it is dumped on non specific areas such as soil, water, sediments and causes serious environmental pollution (Forget, 1993; Satish *et al.*, 2017).

The World Health Organization estimates that 500,000 pesticides poisoning cases occur annually in the world and that 1% are fatal (5000 death/year). According to the Ministry of Commerce, Government of India, Malathion residues were found exceeding the MRL (5 ppb) (Ratna *et al.*, 2012). The Malathion is a wide-spectrum organophosphorus insecticide which is used for agricultural purposes. However, it is toxic to living organisms, including human because it is easily absorbed by the gastrointestinal tract, skin, mucous membranes, and lungs (Inderjeet *et al.*, 1997; Jegadeesh *et al.*, 2010).

Recently, the use of microbes to degrade or remove pesticides has emerged as a powerful technology for pesticide remediation. There are many investigation reports of different organisms like bacteria, algae, yeasts, fungus and plants, characterized in relation to their genome and the enzymes that they produce, that can be utilized for waste treatment or bioremediation of soil and water (Satish *et al*., 2017).

Enzymatic detoxification of organophosphate pesticides by some bacterial species has been reported (Mulbry, 2000; Kim *et al.*, 2005). *Pseudomonas, Bacillus, Flavobacterium, Arthrobacter and Xanthobacter* is some of the bacterial genera isolated from soil, which can degrade insecticides in liquid media.

As the microbial degradation is considered to be a major factor for determining the fate of the pesticides in the environment, the current study was carried out to investigate the ability of bacterial isolates to tolerate and degrade pesticides. Thus, the bioremediation is the only way to minimize such problem concerned with pesticide toxicity in agricultural land. So, the use of *Bacillus* sp strains isolated from cultivating region will be helpful to make ecotoxic free agriculture practices by degrading the pesticide. The genetically and microbiological characterization of the bacteria has been assayed. The ability of isolates to degrade pesticide and using them as carbon and energy sources were evaluated.

II.RESEARCH METHODOLOGY

2.1 Collections of soil samples

The organophosphate pesticide enriched agricultural soil samples were collected from different locations in and around Namakkal area, where like ladies finger, brinjal, tomato, groundnut, maize were cultivated by spraying Malathion. The serially diluted soil samples were spread on Mineral Salt, Medium (MSM) plates containing with malathion (100mg/lit) and were incubated at 37°C for 48 hrs. Any isolate which grows on the MSM agar plate were confirmed as pesticide tolerates. The well isolated colonies were grown on sterile nutrient agar slants as pure cultures. The colony characters were identified based on the colony morphology and staining and biochemical characters.

2.2 Selection of potential isolates

This analysis was performed in 250 mL conical flask containing 100 mL of MSM supplemented with sucrose (10.0 g−lit) and peptone (2.0 g−lit). Sterilized Malathion (100 mg-lit) was added after autoclaving the MSM medium (Yang *et al*., 2006). The one percent of each inoculum was added in a separated flask and incubated at 30°C for 7 days. The biomass concentration was estimated by optical density measurement in 1 cm cuvettes at a wavelength of 660 nm using UV-visible spectrophotometer.

2.3 Optimization of Physicochemical Condition

The growth efficacy of isolates in pesticides was studied at different temperature (25°C, 35°C, 45°C and 55°C), carbon source (Lactose, Dextrose, Fructose, Mannose and Galactose), nitrogen (Peptone, Beef extract, Yeast extract, Malt extract and Casein) source, pH (4, 5, 6, 7 and 8) and incubation time (24hrs, 48hrs, 72hrs, 96hrs, 120hrs) were utilized for optimization of physical condition. The level of degradation was determinate by above said methods.

2.4 Extraction of enzyme and Organophosphorous phosphatase (OPH) assay

Microbial OPH activity was measured spectrophotometrically by monitoring the production of p-nitrophenol. One unit of enzymatic activity was defined as the amount of enzyme capable of catalyzing the production of 1 µmol of 4-nitrophenol per minute under the standard conditions. The protein content of enzyme solution was determined using the method described by Lowry (Maheshwari *et al*., 2017),

III.RESULTS AND DISCUSSION

Agricultural productivity is increased by using pesticides, but only 1% percentage of pesticide is sufficient to kill the pests remaining pesticide enter to the environment and causes different types of pollutions which affect human health also (Battaglin and Fairchild, 2002). Thus, some of the persistent pesticides were banned and some are modified without damaging to the environment. Among different pesticides, organophosphorus pesticide has been extensively used in world agriculture to manage pests or insects of many important crops (Chen *et al*., 2002).

Nowadays different methods have been developed in order to reduce the effects of pesticides on the environment and health, for remediation of contaminated sites and for the treatment of pesticide residues. Many microorganisms that are able to degrade organophosphate pesticide have been isolated from soil around the world. Several bacterial strains capable of degrading organophosphorus compounds have been isolated from pesticide-contaminated soils (Singh, 2006). Previous research has shown that pesticide Malathion, Chlorochlorpyrifose, Dichlorvos degrading bacteria applied as single strains or as consortia to increase the rate of degradation of pesticide Malathion, Chlorochlorpyrifose, Dichlorvos in soil (McCoy *et al*., 2012; Qi *et al*., 2012; Yonar *et al*.,2014).

In the present study, 22 bacteria were isolated from contaminated soil with a history of pesticide application. The isolates showed visible growth on MSM supplemented with 100 mg/ml Malathion, which was utilized it as the sole source of carbon. Bacterial degradation and utilization of malathion compounds have been reported by (Soni *et al*., 2015). In 2016 Sara *et al*., were observed the bacterial isolates from pesticide containing soil samples.

The tentative identification of isolates was done by various biochemical tests. Among the 22 isolates, *P.aeruginosa* was predominant isolate and second most was *B.cereus* and followed by *Serretia marcescens* and least was *B.subtils* (Fig 1). These isolates were tested for the pesticide tolerance against Malathion (100 mg/mL). The growth curves of all isolates were measured by spectrophotometry analysis. The highest tolerant isolates were selected for the optimization of physicochemical parameter for maximum growth. The maximum growth rate of SI13 (*Bacillus cereus*) SI 14 (*Bacillus* sp) and SI 16 (*Serretia marcescens*) were observed and subjected to the optimization process.

Media are optimized or improved if it shows enhanced performance regarding the production of the industrially important economically viable compound. Vijayalakshmi and Usha, (2012) studied the effect of various factors (pH, temperature, carbon and nitrogen sources) for the improved degradation of chlorpyrifos (cps). The present study also aims to investigate the effect with above mentioned parameters on the growth response of the selected bacterial isolates.

While using various temperatures, the maximum growth rate of bacteria was recorded at 35°C followed by 45°C. Among the 3 isolates, SI14 has highly utilized the pesticides effectively and showed maximum growth (Fig 2). This result was almost similar to the previous study of Ahmed *et al*., 2015. They were observed most efficient biodegradation at 30°C. In case of various pH ranges, better results were observed from pH 7 followed by pH 6 (Fig 3).

The degradation patterns of the isolates were also monitored in the presence of carbon and nitrogen stimulants. The degradation is greatly affected by the addition of different carbon sources at variable concentrations. The carbon sources cause the change in the yield of the end product by affecting the rate at which the carbohydrates are metabolized (Abdullah *et al.,* 2003). The highest bacterial growth was observed when using Fructose and followed by Mannose. Current results are contrary to previous studies of (Kavi and Reetha, 2012). They have observed the better growth when using Dextrose than Fructose (Fig 4).

In the case of nitrogen source, growth of bacteria was better in the presence of Peptone, especially SI14 isolate showed the good result (Fig 5). In 2012 Kavi and Reetha were observed maximum growth when using Malt extract and followed by peptone. (Ahmed *et al*., 2015), also observed the better growth on yeast extract containing media. While incubating with various incubation times, better result was recorded with 168hrs, when increasing the incubating time, the bacterial growth rate was reduced (Fig 6). The SI14 (*Bacillus pumilus*) was utilized for determination of MIC of the bacterial isolate with five different concentrations, such as 100mg, 150mg, 200mg, 250mg and 300mg. Among them, 300mg concentration of pesticide containing media has not allowed the growing the colony, therefore 250 mg of concentration as a MIC for SI14 isolate.

In this present study, the potential isolate of SI14 (*Bacillus pumilus*) was used for the production of the organophosphorus enzyme by the OPP assay was found to be present in the intracellular fractions of the isolate. The extracellular OPP activity was 51.04U/ml in a crude sample. The specific activity was increased from ammonium sulfate to dialyze (Table 1).

Bacterial enzymes, such as OPH, MPH, and OpdA, are responsible for the preliminary hydrolysis of organophosphates and OPH (organophosphorus hydrolase) has been the most widely studied for its hydrolytic activity on organophosphates. In 2015, Soni *et al.*, isolate the OPH producing *Pseudomonas aeruginosa* from the pesticide contaminate area. In 2016, Sara *et al.,* observed the organophosphorus compounds degrading *Bacillus licheniformis* from soil samples.

The present study was concluded the isolation of a bacterial consortium which is capable of utilizing malathion as a source of carbon. Usage of xenobiotic compounds by soil microorganisms is an important phenomenon by which these compounds are removed from the environment, thus avoiding environmental pollution. The results of the present study suggest that the *Bacillus pumilus*, which was able to grow in medium in the presence of pesticide and therefore, be used for bioremediation of pesticide contaminated soil.

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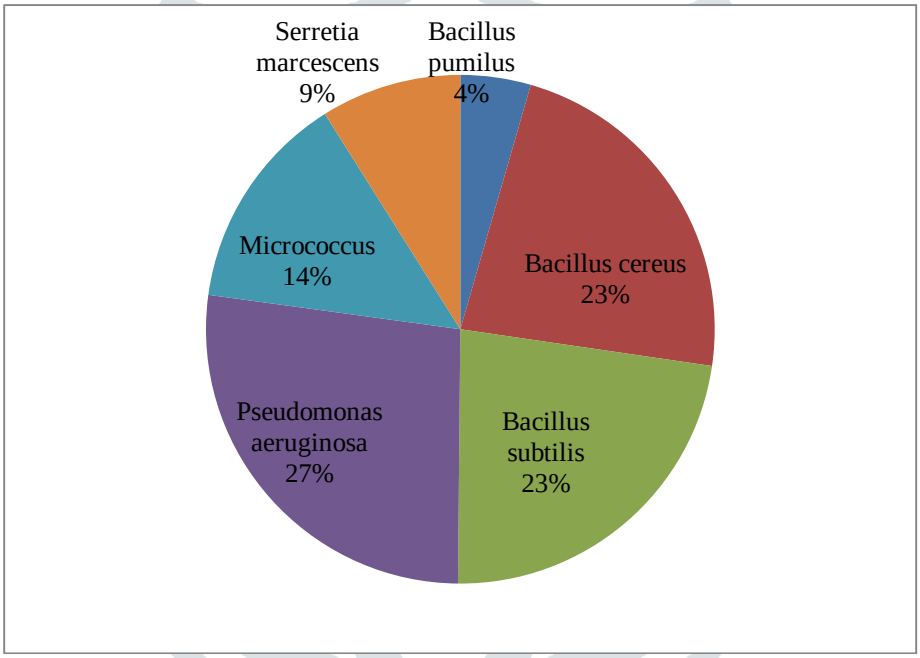

Fig. 1

Percentage incidence of Bacterial species on soil samples

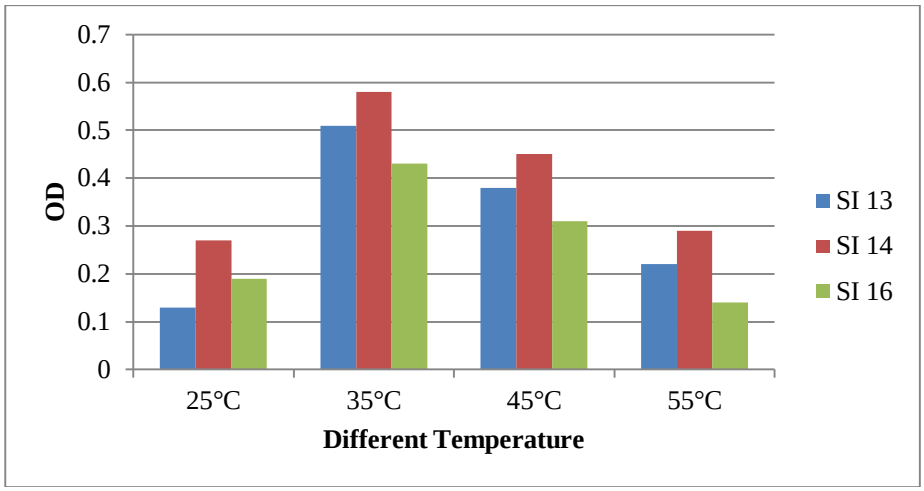

Fig.2
Effect of temperature for the growth of Malathion degrading bacteria

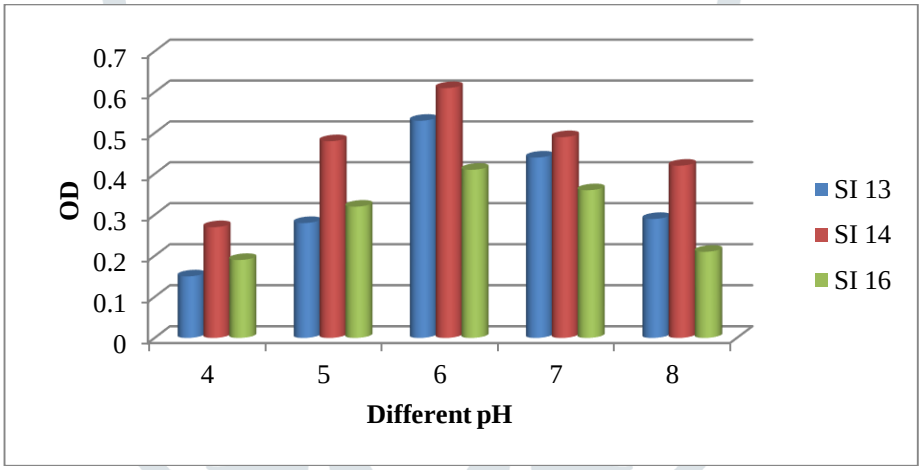

Fig 3
Effect of pH for the growth of Malathion degrading bacteria

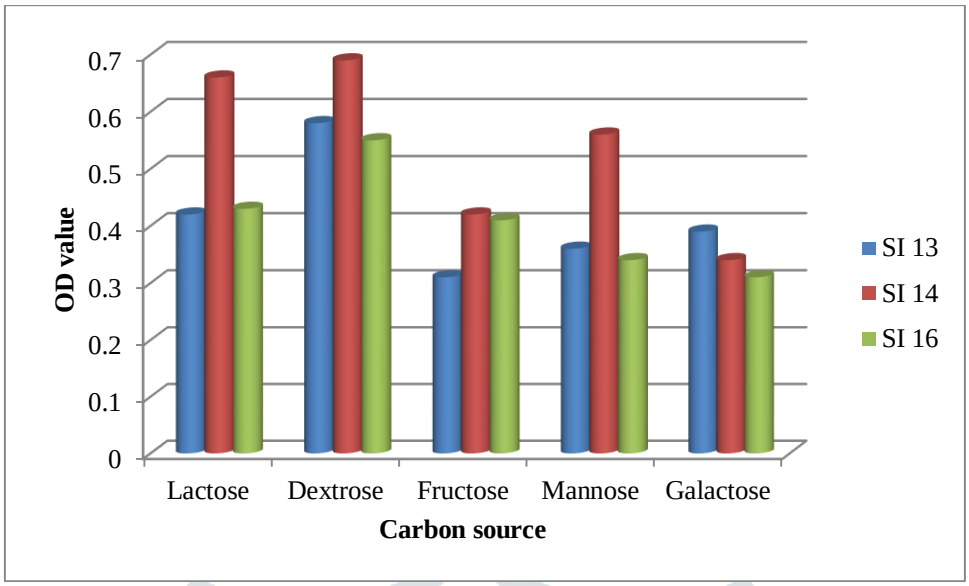

Fig .4
Effect of Carbon source for the growth of Malathion degrading bacteria

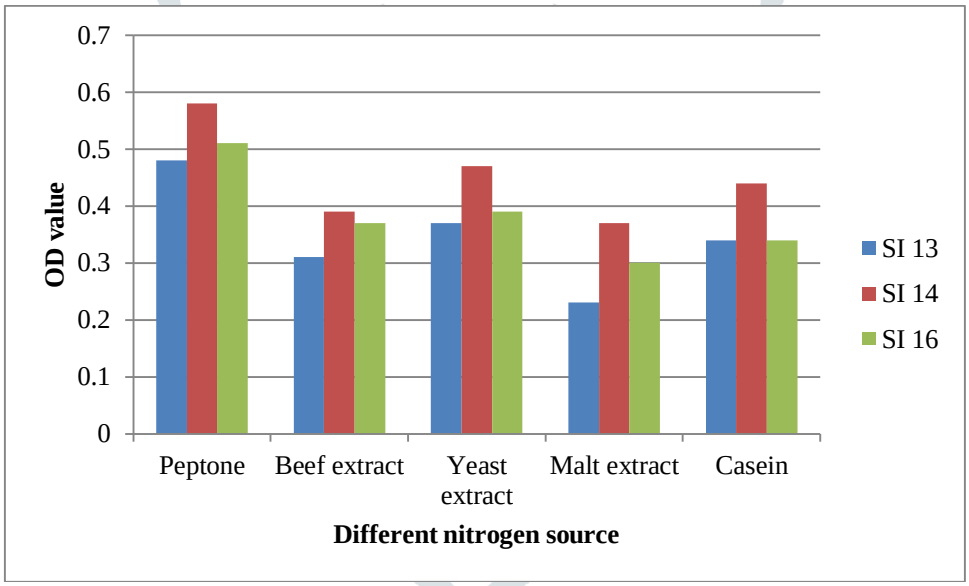

Fig.5
Effect of Nitrogen source for the growth of Malathion degrading bacteria

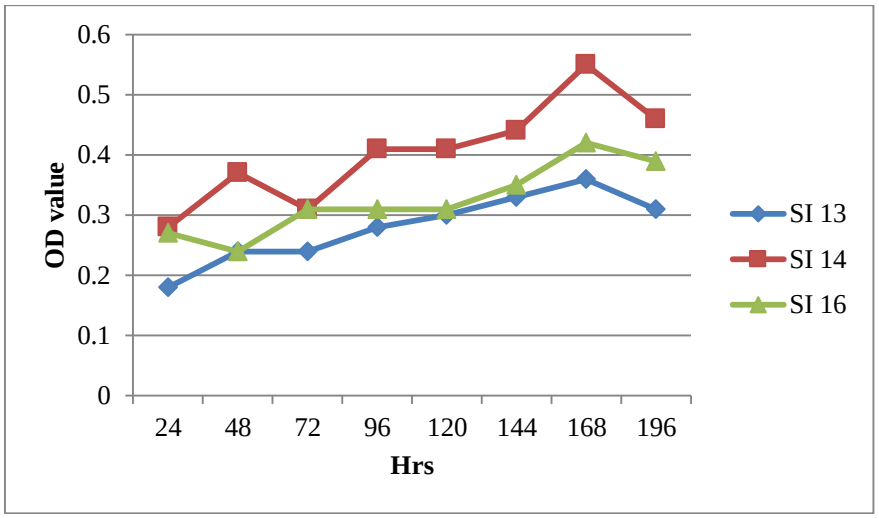

Fig. 6
Effect of Incubation time for the growth of Malathion degrading bacteria

Table 1
Production of and purification of and Organophosphorous Phosphatase

S. No	Purification steps	Enzymes activity	Protein con. (mg/ml)	Specific enzyme activity(U/ml)	Purification fold (%)
1.	Crude	17.5	1.2	14.58	1
2.	Ammonium sulphate	21.3	0.81	26.29	1.80
3.	Dialysis	24.5	0.48	51.04	1.94