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Original Research Article

Biodegradation of Quinolphos insecticide by *Pseudomonas* strain isolated from Grape rhizosphere soils

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A B S T R A C T

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This study was aimed to determine the degradation potential of *Pseudomonas* species isolated from grape rhizosphere soils towards the organophosphorus insecticide Quinalphos. A total number of 14 *Pseudomonas* strains have been isolated and screened for their tolerance level against various concentrations of Quinalphos (5mg/L, 10mg/l, 15mg/L and 20mg/L). Results indicated that out of 14 isolated *Pseudomonas* strains, only one strain could tolerate and degrade the highest concentration of Quinalphos. This strain was subjected to degradation of insecticide at the level of 15mg/L and 20mg/L.The results showed that in the presence of glucose, isolated strain could degrade Quinalphos up to 90.4%, whereas up to 38.2% in the absence of glucose, which may be due to the role of glucose as an inducer for the growth of organism.

Introduction

In developing countries like India with agriculture based economy, there is increasing trend of cash crop cultivation like grapes and is increasing particularly in Maharashtra state. In India, productivity is highest among the grape growing countries of the world, because of special arbour training systems are provided for grape cultivation. But large complex of insect pests ranging from borers to root feeding insects are responsible for heavy losses of commercial crops like grapes (as a perennial crop) both quantitatively and qualitatively.

Pests, pathogens and weeds are important factors limiting the productivity and quality of grapes. In view of this, several agrochemicals are continuously used to protect the grape wine yards. In order to reap maximum yields of grapes, the farmers resort to more insecticidal application to combat the pest problem (Dollacker, 1991). But, the extensive use of these agrochemicals leads to an accumulation of a huge amount of residues in the environment. Therefore it causes a substantial environmental health hazard due to uptake and accumulation of these toxic compounds in the food chain and

drinking water (Mohammed, 2009). Among the insecticide use, organophosphorus insecticide that is quinalphos at the top list to control insects such as flea beetles, thrips, mealy bugs, leaf hoppers on grapes.

The major environmental concern in the use of organophosphorus insecticides is their capacity to leach from soil and contaminate the ground water(Kookana et.al.1998) or if immobile, they would persist on the top soil where it could accumulate to toxic levels in the soil and become harmful to microorganisms, plants, wildlife and man(Amakiri, 1982). Organophosphate pesticides are a group of highly toxic heterogeneous compounds that share structurally, a phosphoric acid derivative which is widely used for plant protection and pest control. There are currently 140 organophosphate compounds being used as pesticides and as plant growth regulators around the world (Kang et al., 2006), which are components of more than 100 types of commercially available pesticides (such as Paraoxon, Parathion, Malathion, Diazinon, Quinolphos and Dichlorvas).

Most synthetic organophosphate compounds are widely used as insecticides in agriculture. These compounds are powerful inhibitors of acetylcholinesterase, a vital enzyme involved in neurotransmission, in the form of acetylcholine substitutes (Bakry et al., 2006) and also causes various clinical effects (Serdar and Gibson, 1985; Grimsley et al., 1998). There are many conventional methods employed for the remediation of organophosphate contaminated sites mainly chemical treatment, recycling, pyrolysis, incineration and landfills but these are less efficient and costly and can also lead to the formation of toxic intermediates (Dua et al., 2002; Richins et al., 1997). So, for this reason, many bacteria that are able to degrade organophosphate pesticide have been isolated from soil around the world (Zhongli et al., 2001; Chang et al., 2005; Horne et al., 2002).

American academy of Microbiology defined bioremediation as the use of living organisms to reduce or eliminate environmental hazards resulting from accumulations of toxic chemicals or other hazardous wastes. Bioremediation methods to treat xenobiotic compounds like insecticides in soil have gained considerable attention due to their ecofriendliness and they have been used successfully in many countries. So, isolation of indigenous bacteria from insecticide contaminated soil capable of metabolizing organophosphate compounds has received considerable attention (Richins et al., 1997; Mulchaldini et al., 1999).

Enzymatic detoxification [phosphotriesterase (PTE)] of organophosphate pesticides by some bacterial species has been reported (Mulbry et al., 1986; Mulbry, 2000; Chen-Goodspeed et al., 2001; Kim et al., 2005). *Pseudomonas, Bacillus, Flavobacterium, Arthrobacter and Xanthobacter* are some of the bacterial genera isolated from soil which can degrade insecticides in liquid media (Gossel and Bricker, 1994).

Thus, the bioremediation is the only way to minimize such problem concerned with pesticide toxicity in agricultural land like grape wine yards. So, use of *Pseudomonas* strains isolated from grape rhizosphere region will be helpful to make ecotoxic free agriculture practices by degrading the insecticide Quinalphos.

Materials and Methods

Chemicals and Media

Standard analytical grade solution of Quinalphos (o,o-diethyl, o-quinoxalin-2-yl-phosphorothioate) (25% E.C.) was purchased from the local market of Sangli, (M.S.), India. A liquid preparation of Mineral Salts Medium (MSM) containing 0.3% $NaNO_3$, 0.05% $MgSO_4$, 0.05% KCl, 0.1% K_2HPO_4, 0.05% KH_2PO_4, 0.001% $FeSO_4$, 0.05% yeast extract and 1.0% glucose. Nutrient broth and Nutrient Agar were used for the screening of cultures and Quinalphos resistance.

Collection of Soil Samples

About 20 soil samples were collected from grape wine yards of seven different locations of Sangli district (Maharashtra), to isolate Quinalphos insecticide resistant bacteria. These grape wine yards have been sprayed with Quinalphos for past few years. Soil samples were collected at different sites of each field by using sterile scalpel and these soil samples were transferred to sterile polythene bags and used for analysis.

Enrichment, isolation and screening of bacterial strains

The bacterial cultures capable of degrading Quinalphos were isolated from collected soil samples using enrichment technique, with varying concentrations of Quinalphos (5mg/L, 10mg/L, 15mg/L and 20mg/L) in the Mineral Salts medium. The soil sample (1gm) was inoculated in to 100ml of mineral salt medium supplemented with above various concentrations of Quinalphos in 500ml Erlenmeyer flasks. The flasks were incubated on a rotary shaker at 150 cycles per minute for 7 days at room temperature (25-30^oC). At daily intervals, one loop full of enrichment culture from the flask was streaked on nutrient agar plates supplemented with Quinolphos (5-20mg/L) and incubated at room temperature for 24-48hrs.

Individual colonies of bacteria which varied in shape and color were picked up and were subcultured onto nutrient agar plates containing same concentration of Quinalphos until pure culture was isolated. The isolated strain was maintained at 4^oC and subcultured after every three months. The bacterial isolates were identified on the basis of classification schemes published in Bergey's Manual of Systematic Bacteriology (Krieg and Holt, 1984).

Biodegradation of Quinalphos

To study the degradation of Quinalphos, the isolated strain WL DumpQ10 was grown in the mineral based medium as mentioned above with and without glucose and with 10mg/L concentration of Quinalphos as the sole source of carbon and nitrogen. The flasks were incubated on rotary shaker at 150 rpm at room temperature.

The contents of the flasks were checked by taking 5ml of culture drawn from mineral based medium and centrifuged at 5000 rpm for 10 min. The pellet was discarded and the supernatant was analyzed by UV-Visible spectrophotometer to determine the Quinalphos degradation by isolate WL DumpQ10. Degradation study was conducted for every 2 days interval up to 8 days. The percent degradation of Quinalphos by isolate WL DumpQ10 was also determined.

Optimal conditions for degrading Quinalphos by WL DumpQ10

To determine the optimal conditions for degrading Quinalphos by WL DumpQ10, single factor test was designed in this study under different conditions. To confirm the effects of temperature on degradation, the degradation broth media were placed at various temperatures as 10, 20, 25, 30, 35 and 40^0C. The media were prepared at P^H values from 4.0 to 11.0 buffers, for the measurement of the effects of the P^H on degradation. All the experiments were conducted in triplicates. The non-inoculated controls throughout the studies were implemented at the same condition in order to exclude the abiotic degradation affection.

Extraction of the metabolites and GCMS analysis

After 8 days of incubation, the broth was centrifuged at 10000 rpm for 15 min. The supernatant obtained was used to extract metabolites with ethyl acetate (1:1). The extracts were dried and evaporated to dryness in an evaporator. The obtained residue was dissolved in small volume of methanol and used for GCMS analysis.

The extract was analyzed by Gas Chromatography coupled with mass spectroscopy. Gas chromatography was performed in temperature programming mode with a DB 530-m fused silica capillary column (0.25mm, i.d. x 0.25 μm film thickness) attached to a mass spectrophotometer. Samples were injected in to a split mode temperature program of 180^0C for 1.5 min, 260^0C for 20 min, at the rate of 10^0C/min. injector temperature was 260^0C and detector temperature was 280^0C. Nitrogen was used as carrier gas. The compounds were identified on the basis of mass spectra and were compared using National Institute of Standards and Technology (NIST) library.

Results and Discussion

Since Quinalphos is one of the most commonly used commercial insecticide on grape wine yards, (Kuperberg, et.al., 2000), it is therefore logical that the bacteria from Quinalphos contaminated grape wine yards could be able to degrade this insecticide. Due to excessive use of these organophosphorus insecticides on grapes rise many hazardous effects in the environment. By considering toxicity of compound it is essential to remove them from environment. Biological removal of insecticide is the easiest way, as the soil microorganisms can use such hazardous compound and convert them into non-toxic metabolites.

Isolation and Characterization of Quinalphos Degrading Bacterium

After repeated enrichment and purification processes, we obtained approximately 14 strains of organisms with different colony morphologies from collected grape wine yard soils. But the degradation experiment showed the isolate WL DumpQ10 possessed relatively higher degradation capacity of degrading quinolphos (20mg/L) by 90.4% after incubating 8 days at P^H 7.0 and room temperature about 30^0C. WL DumpQ10 utilized Quinalphos as its sole carbon and energy source in Mineral salt Medium. Thus, strain WL Dumpq10 was selected for further detail investigation.

On the basis of their morphological and biochemical characteristics, the cultures were confirmed as belong to the Genus *Pseudomonas*, according to Stolp and Gadakari(1981) and Bergey's Manual of

Systematic Bacteriology. The selected isolates were studied for their tolerance level against Quinalphos. Results indicated that out of 14 selected isolates, only one isolate (WL DumpQ10) was capable of tolerating the highest concentration of Quinalphos tested in the present study. The growth profile of this selected isolate showed that the maximum growth was achieved after 24-30 hrs of incubation in insecticide amended medium.

Pseudomonas strain used (WL DumpQ10) in this study had shown a range of degradation capability. Among the two media composition tested, mineral salts medium with glucose showed better degradation environment towards Quinalphos than mineral salts with insecticide alone.

Effect of Temperature and p^H on Quinalphos Degradation in MSM

Quinalphos was degraded by WL DumpQ10 during incubation temperatures ranging from 10^0C, to 40^0C. The Quinalphos residues were detected after 8 days treatment. In cultures incubated at higher temperatures 30^0C and 35^0C, the degradation rate reached 91.2% and 85.4% within 8 days, but degradation rate was low at any other temperature.

The p^H is also an important factor which significantly affects the degrading ability of bacteria capable of degrading toxicities. To determine the effect of p^H on degradation, MSM medium prepared with different p^H buffers, fortified with 20mg/L Quinalphos and incubated at 30^0C. Eight different p^H (4.0, 5.0, 6.0, 7.0, 8.0, 9.0, 10.0, and 11.0) were tested in the optimization experiment. The optimal initial p^H value for degradation was between 6.0 and 8.0.

Identification of Quinalphos degradation metabolites

The degradation metabolites of Quinalphos by WL DumpQ10 was extracted and identified by GC/MS using NIST library. Upon GC/MS analysis Quinalphos shows retention time of 11.217min. This analysis of the metabolites showed the presence of three products. This result observed after degradation of Quinalphos by isolated Pseudomonas strain obtained metabolites as compared to the studies previously described. The results obtained from GC/MS analysis show the formation of [2, 5- cyclohexadiene-1, 4-dione, 2, 6-bis (1, 1-dimethylethyl)-], butylated hydroxy toluene, pentadecanoic acid. The biological treatment of chemically contaminated soil involves the transformation of complex or simple chemical compounds into non-hazardous forms (Naveen et. al., 2011). In the light of this fact, biodegradation, especially microbial degradation has proven to be a suitable method for insecticide elimination. Previous studies indicated that soil bacteria play important roles in degrading and detoxifying organophosphorus insecticide residues in the environment. Reports showed that *Rhodobacter sphearoides* could effectively degrade pesticides like 2,4- D and Quinalphos (Chalam et. al., 1996). The higher degradation capacity in glucose amended medium may be due to co-metabolism, where addition of easily metabolized organic matter such as glucose increases biodegradation of recalcitrant compounds that are usually not used as carbon and energy sources by microorganisms (Prescott et.al., 2002). Previous reports suggested that the use of glucose as co-substrate had increased the rate of biodegradation (Swaminathan and Subrahmanyam, 2002).

Figure.1 Optimal conditions for degrading Quinalphos by WL DumpQ10. a) Effect of temperature on the degradation of Quinalphos by WL DumpQ10; b) Effect of p^H on the degradation of Quinalphos by WL DumpQ10

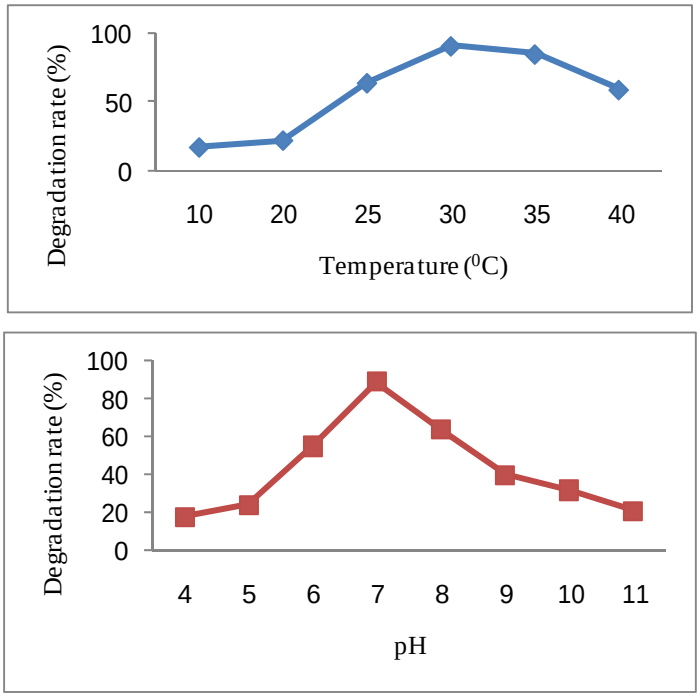
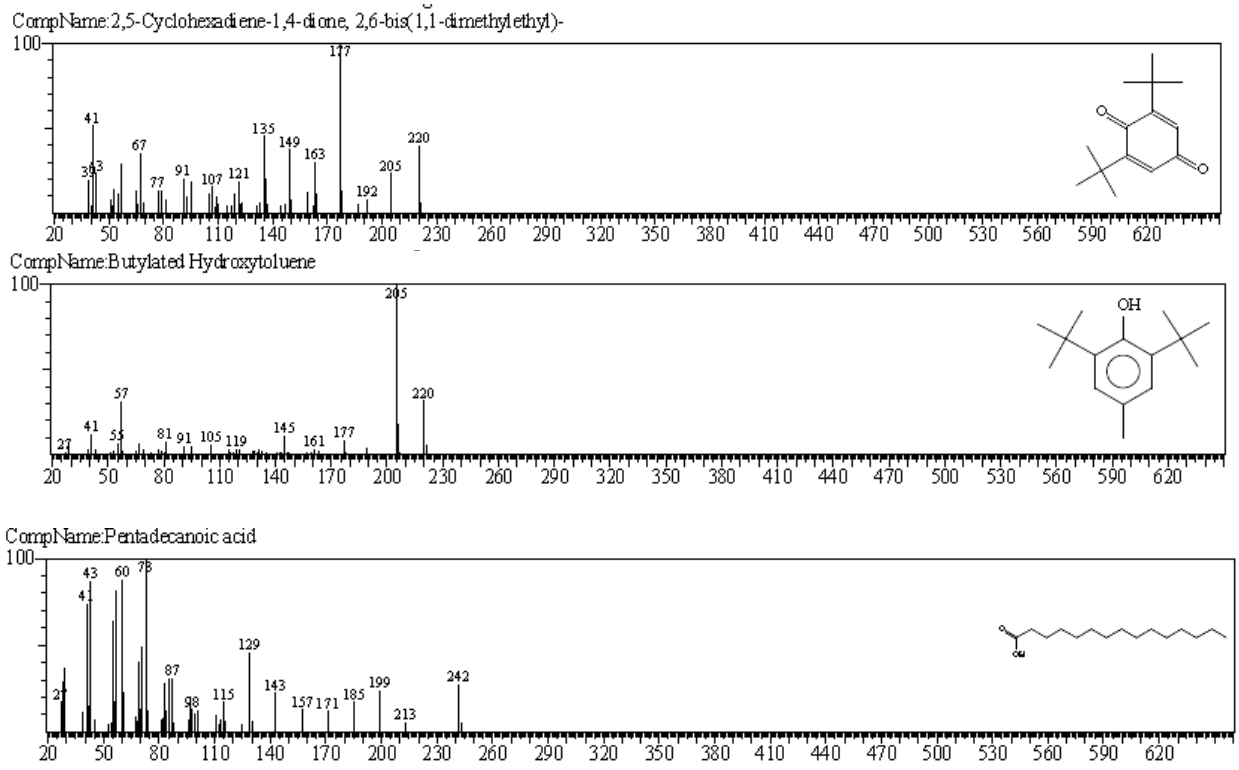


Figure.2 Identification of Quinalphos degradation metabolites



Also earlier studies suggested that, many soil applied pesticides are degraded more rapidly following repeated application at the same site (Racke and Coats, 1990)

In conclusion our results indicated that *Pseudomonas* strain could be a good choice for the bioremediation of Quinalphos contaminated soil and water. *Pseudomonas* is a versatile genus and also according to previous studies this genus could degrade a number of chemicals like pesticides including carbaryl (Vandana and Phale, 2005), malathion, paranitrophenol. I is widely present in soil and can be used to clean up different man made xenobiotic compounds.

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