Journal of Hazardous Materials 174 (2010) 295–298

Contents lists available at ScienceDirect

Journal of Hazardous Materials

journal homepage: www.elsevier.com/locate/jhazmat

Biodegradation of propargite by *Pseudomonas putida*, isolated from tea rhizosphere

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ARTICLE INFO

Article history:
Received 3 March 2009
Received in revised form 9 September 2009
Accepted 10 September 2009
Available online 17 September 2009

Keywords:
Tea
Propargite
Pseudomonas
Biodegradation
Glucose

ABSTRACT

Biodegradation of miticide propargite was carried out *in vitro* by selected *Pseudomonas* strains isolated from tea rhizosphere. A total number of 13 strains were isolated and further screened based on their tolerance level to different concentrations of propargite. Five best strains were selected and further tested for their nutritional requirements. Among the different carbon sources tested glucose exhibited the highest growth promoting capacity and among nitrogen sources ammonium nitrate supported the growth to the maximum. The five selected *Pseudomonas* strain exhibited a range of degradation capabilities. Mineral salts medium (MSM) amended with glucose provided better environment for degradation with the highest degradation potential in strain SPR 13 followed by SPR 8 (71.9% and 69.0% respectively).

1. Introduction

Tea (*Camellia sinensis* (L) O.Kuntze), being a perennial crop, provides a stable environment for a number of pests and diseases. Tea plantations suffer heavily from the infestation of several of them. Pests, pathogens and weeds are important factors limiting the productivity and quality of processed tea. To protect the crop from various pests and pathogens, a number of agrochemicals are used. The main concern of indiscriminate use of these agrochemicals is their capacity to leach from soil and pollute the ground water [1] or if immobile, they would persist on the top soil [2] where it could accumulate to toxic levels in the soil and become harmful to microorganisms, plants and man [3]. Bioremediation is a process which uses live organisms to decrease environmental hazards resulting from accumulation of lethal chemicals or other hazardous wastes [4]. Bioremediation is becoming gradually more adaptable due to its ecofriendliness and is one of the most cost effective methods compared to physical and chemical remediation methods [5,6]. *Pseudomonas*, *Flavobacterium*, *Arthobacter*, *Xanthobacter* are some of the species isolated from soil which can degrade pesticides in liquid media [7–9].

In the present study, degradation of a non-systemic acaricide propargite (2-[4-(1,1-dimethylethyl) phenoxy] cyclohexyl 2-propynyl sulfite) was studied. Propargite is widely applied for controlling a variety of phytophagous mites on many crops including cotton, vines, fruit trees, vegetables, hops and nuts, etc. [10]. In tea plantations, it is used in controlling red spider mites [11]. Propargite is an irritant in humans and may also be a sensitizer, as several outbreaks of dermatitis was observed in field workers exposed to this compound [12] and WHO has classified propargite as "slightly hazardous" [13]. Propargite is a high priority pesticide for evaluation as a toxic air contaminant, in a ranking in which it tied with cyanazine for first place [14]. It is highly toxic to fish and crustaceans [15]. Propargite is moderately persistent and immobile in soils. In water, it hydrolyzes rapidly (half-life 2.2 days) under alkaline conditions (pH 9), where as moderately persistent at pH 7 (half-life 75 days), and very persistent at pH 5 (half-life 120 days). As tea soil is acidic (4.8–5), the persistence of the chemical is of great concern. So in the present study, an attempt was made to examine the degradation of this chemical by the tea soil microflora. For degradation studies, *Pseudomonas* sp. was selected, as it is a well known biodegrader of agrochemicals [16–18] and its occurrence was reported earlier in tea rhizosphere [19].

2. Materials and methods

The pesticide used in the current study was reference standard propargite with purity of 95.4%, obtained from Riedel-de-Haen, Germany. The chemical was dissolved in acetone for further studies. In the present study, degradation of the target compound (propargite) only was studied, not its metabolites. The detection and quantification limit ranges for propargite in this study was

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0304-3894/$ – see front matter © 2009 Published by Elsevier B.V.
doi:10.1016/j.jhazmat.2009.09.050

Table 1
Screening of selected isolates on propargite amended medium.

Isolates	5 mg/L	7 mg/L	10 mg/L
SPR1	+++	–	–
SPR2	+++	+	–
SPR3	+++	–	–
SPR4	+++	+++	+++
SPR5	++	++	–
SPR6	+++	–	–
SPR7	+++	+++	+++
SPR8	+++	+++	+++
SPR9	+++	–	–
SPR10	+++	+	–
SPR11	+++	+++	+++
SPR12	+++	–	–
SPR13	+++	+++	+++

(+++) good growth; (++) moderate growth; (+) poor growth; (–) no growth.

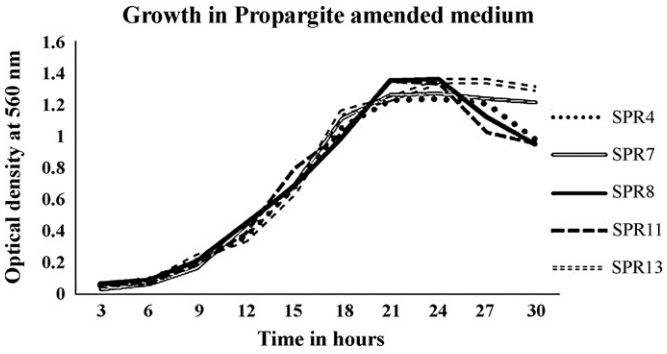

Fig. 1. Growth profile of the selected isolates in propargite amended medium.

0.3 mg/L and 1.0 mg/L respectively. The average recovery percentage at 0.3 mg/L level was 95%.

2.1. Isolation

The bacterial culture capable of degrading propargite was isolated by aerobic shake flask culture in mineral salts medium (MSM) containing propargite (5 mg/L). The medium contained: 200 mg $MgSO_4$, 900 mg K_2HPO_4, 200 mg KCl, 2 mg $FeSO_4$, 2 mg $MnSO_4$, 2 mg $ZnSO_4$, 1000 mg NH_4NO_3/L. It was adjusted to pH 7.0 by 1N HCl and sterilized by autoclaving at 121 °C for 15 min. This medium was inoculated with filtered soil suspension (10% initially and 1%, in subsequent transfer) derived from 10 g tea rhizosphere soil mixed with 150 mL mineral salts medium. Incubation was done at 30–35 °C. After incubation, soil suspension was serially diluted and appropriate dilutions were plated on King's B (KB) agar medium [20] containing propargite (5 mg/L). Isolated colonies were subcultured and purified on KB medium containing propargite.

2.2. Screening

Tolerance levels of the isolates were studied in nutrient agar plates containing different concentrations of propargite (5–10 mg/L), and the isolates exhibiting the highest degree of tolerance were stored in nutrient agar slants containing propargite and stored at 4 °C. The selected isolates were screened for their different nutritional requirements.

2.3. Study of growth profile in pesticide amended medium

The growth profile of the selected isolates on pesticides amended medium was carried out in MSM medium containing propargite (10 mg/L) and observed at every 3 h intervals by using UV–VIS spectrophotometer (Specord® S100, Analytic Jena, Germany) at 560 nm (OD_{560}).

2.4. Nutritional requirements

Effects of different inorganic elements such as carbon and nitrogen sources were studied by incorporating them in the basal medium at 1% level and incubated at 35 ± 2 °C. The growth was observed by measuring the absorbance at 560 nm (OD_{560}). The basal medium used was MSM broth. Identification of the selected isolates was carried out by morphological, biochemical and staining technique as mentioned by Stolp and Gadkari [21].

2.5. Biodegradation study

For degradation study, selected isolates were grown on mineral salts medium containing 10 mg/L propargite in 500 mL baffled flask containing 200 mL MSM media. In a separate experiment, media containing glucose (200 mg/L) and pesticide in MSM media was used for degradation as glucose is easily degradable substance. The cultures were incubated in a rotary incubator shaker (150 rpm) at 30–35 °C for 24–30 h. After achieving the log phase, the cultures were centrifuged at 9000 rpm for 15 min. After separating the organisms, supernatant was taken in a 125 mL separating funnel and extracted with 50 mL of acetonitrile. After partitioning, the extract was evaporated to near dryness at 65 °C using rotary vacuum evaporator and analysed in HPLC (Agilent 1100 series) as per the following conditions: column: Zorbax Rx C18 (4.6 mm × 250 mm), detector: DAD (Diode array detector), wavelength: 225 nm, flow rate: 1.5 mL/min, mobile phase: acetonitrile (ACN) + water (75 + 25), injection volume: 10 μL, final dilution: 10 mL ACN.

3. Results and discussion

A total of 13 strains (denominated as SPR 1 to SPR 13) was isolated by aerobic culture method. These isolated strains were subcultured and purified in KB medium. The selected isolates were studied for their tolerance level against propargite. Results indicated that out of 13 selected isolates, five isolates were capable of tolerating the highest concentration of propargite (10 mg/L), tested in the present study (Table 1). The growth profile of these five selected isolates showed that the maximum growth was achieved after 24–30 h of inoculation in pesticide amended medium (Fig. 1). These five isolates (SPR 4, SPR 7, SPR 8, SPR 11 and SPR 13) were

Table 2
Growth on different carbon sources (OD at 560 nm).

Isolates	Glu.	Galac.	Malt.	Star.	CMC.	Xyl.	Man.	Sorb.	Salic.
SPR4	1.554 ± 0.20	1.175 ± 0.10	0.125 ± 0.02	0.120 ± 0.02	0.286 ± 0.02	0.212 ± 0.02	0.533 ± 0.03	0.298 ± 0.02	0.121 ± 0.01
SPR7	0.975 ± 0.10	0.704 ± 0.03	0.116 ± 0.01	0.136 ± 0.02	0.470 ± 0.02	0.084 ± 0.01	0.513 ± 0.03	0.179 ± 0.01	0.472 ± 0.03
SPR8	0.982 ± 0.10	0.703 ± 0.02	0.370 ± 0.01	0.325 ± 0.02	0.573 ± 0.03	0.351 ± 0.02	0.207 ± 0.01	0.292 ± 0.01	0.198 ± 0.01
SPR11	1.004 ± 0.11	0.776 ± 0.03	0.137 ± 0.01	0.203 ± 0.01	0.392 ± 0.02	0.252 ± 0.02	0.426 ± 0.02	0.274 ± 0.01	0.377 ± 0.02
SPR13	0.986 ± 0.10	0.764 ± 0.03	0.267 ± 0.01	0.343 ± 0.01	0.547 ± 0.03	0.288 ± 0.02	0.227 ± 0.01	0.422 ± 0.02	0.318 ± 0.02

Glu: glucose; Galac: galactose; Malt: maltose; Star: starch; CMC: carboxyl methyl cellulose; Xyl: xylose; Man: mannose; Sorb: sorbitol; Salic: salicin.

Table 3
Growth on different nitrogen sources (OD at 560 nm).

Isolates	$(NH_4)_2SO_4$	NH_4NO_3	NH_4Cl	$NaNO_3$	NH_2CONH_2	KNO_3
SPR4	0.667 ± 0.04	1.559 ± 0.10	1.041 ± 0.17	0.589 ± 0.01	1.012 ± 0.10	0.769 ± 0.04
SPR7	0.660 ± 0.02	0.990 ± 0.10	0.912 ± 0.20	0.697 ± 0.03	1.085 ± 0.12	0.788 ± 0.02
SPR8	0.796 ± 0.10	0.992 ± 0.30	1.045 ± 0.15	0.482 ± 0.02	0.706 ± 0.02	0.759 ± 0.03
SPR11	0.714 ± 0.10	1.130 ± 0.20	1.057 ± 0.19	0.762 ± 0.06	1.029 ± 0.12	0.684 ± 0.03
SPR13	0.629 ± 0.03	1.121 ± 0.25	0.943 ± 0.15	0.675 ± 0.04	0.835 ± 0.07	0.744 ± 0.05

$(NH_4)_2SO_4$: ammonium sulfate; NH_4NO_3: ammonium nitrate; NH_4Cl: ammonium chloride; $NaNO_3$: sodium nitrate; NH_2CONH_2: urea; KNO_3: potassium nitrate.

studied for their nutritional requirements. Among the different carbon sources, glucose showed the highest growth promoting capacity followed by galactose (Table 2). Among different nitrogen sources, ammonium nitrate exhibited highest growth promoting capability (Table 3). The five selected *Pseudomonas* strains used in propargite biodegradation study had shown a range of degradation capability. Among the two media compositions tested, mineral salts medium with glucose provided better degradation environment towards propargite than mineral salts with pesticide alone. Out of the five strains tested for their degradation efficiency, strain number SPR 13 showed the highest level of degradation followed by SPR 8. Strain number SPR 13 degraded propargite in glucose amended mineral salts medium up to 71.9% in 24 h whereas in mineral salts medium its efficiency declined to 32.0%. Strain number SPR 8 could degrade propargite up to 69.0% in the presence of glucose but 37.1% in the mineral salts medium (Table 4). Strain number SPR 4 and SPR 11 could degrade propargite to 58.8% and 51.0% respectively in presence of glucose whereas a mere 13.7% and 16.8% in MSM medium alone. The lowest degradation capacity was observed with strain number SPR 7. Generally, the major degraded products of propargite are carbon dioxide, propargite glycol ether, and p-tertiary butylphenoxy cyclohexanol [22]. The higher degradation velocity in glucose amended medium indicated that glucose played a crucial role in initial growth of the bacteria. It 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 [23]. Previous reports suggested the use of glucose as co substrate increased biodegradation rate [24,25]. This process of co-metabolism is finding widespread applications in biodegradation management [26,27]. Degradation of propargite by isolated tea rhizosphere microflora showed that these organisms had adapted to this chemical because of repeated applications in field. Earlier studies suggested that, many soil applied pesticides are degraded more rapidly following repeated application at the same site [28]. On the basis of the isolates morphological and biochemical characteristics, the cultures were found to belong *Pseudomonas putida*. *Pseudomonas* is a versatile genus and previous reports suggested that this genus could degrade a number of chemicals like pesticides including carbaryl [29], malathion [30], *p*-nitrophenol and parathion [31] and bethoxazin [32] and present study also supported their immense nutritional diversity.

Table 4
Biodegradation of propargite (10 mg/L).

Isolates	% degradation	
	Glucose[a]	Glucose[b]
SPR4	58.8 ± 5.45	13.7 ± 1.24
SPR7	34.3 ± 2.76	14.8 ± 1.56
SPR8	69.0 ± 6.34	37.1 ± 2.69
SPR11	51.0 ± 5.28	16.8 ± 2.10
SPR13	71.9 ± 7.15	32.0 ± 2.14

[a] Glucose present.
[b] Glucose absent.

Acknowledgements

The authors are thankful to Dr. N. Muraleedharan, Adviser and Dr. P. Mohan Kumar, Director of UPASI Tea Research Institute for their constant encouragement and support.

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