ScienSage

Journal of Advanced Scientific Research

Available online through http://www.sciensage.info

ISSN
0976-9595
Research Article

BIODEGRADATION OF DICHLORVAS BY STRAIN OF *PSEUDOCHROBACTRUM SACCHAROLYTICUM* FROM GRAPE WINE YARD SOIL

G. V. Mali*[1], K. R. Pawar[2]

Dept. of Microbiology, BharatiVidyapeeth's MBSK KanyaMahavidyalaya, Kadegaon, Dist. Sangli, Maharashtra, India
V.N. Arts, Commerce and B. N. Science Mahavidyalaya , Shirala Dist. Sangli, Maharashtra, India
**Corresponding author: gajamali@rediffmail.com*

ABSTRACT

Dichlorvas is extensively used for controlling insect pests on grape wine yards and contamination of soil system in grape wine yards has been reported from all parts of the world. Bioremediation potential of microorganisms provides a suitable way to remove such contaminants from the environment because organophosphate compounds are totally mineralized by the microorganisms. In the present investigation, an indigenous bacterium capable of degrading dichlorvas was isolated from pesticide contaminated soil samples of grape wine yards and identified as a strain of *Pseudochrobactrum saccharolyticum*. It was found to have a potential to degrade it and the metabolites of degradation were butanedioic acid, 2, 3-dihydroxy-dimethyl-ester ; tricyclo (5.3.0.0(2,6)deca-3,10-dione, 6,7-dichloro;and thiacyclopentane-3-ol which were identified by GC/MS analysis using NIST library.

Keywords: Pesticides, Dichlorvas, Biodegradation, Metabolites

1. INTRODUCTION

Among the various groups of pesticides that are currently used worldwide, organophosphorus pesticides(OP)is the major and most widely used group that accounts for more than 36% of the total world market. All organophosphorus pesticides are phosphoric acid esters. They are also called organophosphates and include phenyl, aliphatic and heterocyclic derivatives. They are predominantly used in horticulture especially on grape wine yards. Indian grapes are under constant scrutiny of the environment and health protection agencies worldwide, as they receive large number of organophosphorus pesticides during cultivation.

Among the organophosphates, dichlorvas is extensively used for controlling insect pests of orders Coleopteran, Dipterans, Lepidoptera, Hemiptera on grape yards. Overdose of these insecticides affect the nervous and reproductive system of insects. They also block prolonged activity of the enzyme cholinesterase (ChE), responsible for the nervous impulse in organisms. Owing to the large scale use of organophosphates, contamination of soil system in grape wine yards has been reported from all parts of the world. In light of this, bioremediation or bioremoval potential of microorganisms provides a suitable way to remove contaminants from the environment as, organophosphate compounds are totally mineralized by the microorganisms. The rhizospheric bacteria have developed genetically determined system against toxicants due to their continuous exposure to such environmental stresses [1] .Bacteria play significant roles in the transformation of insecticide, herbicide, and fungicide molecules to various non-toxic degradation products. The transformation mechanisms include oxidation-reduction, conjugation, hydrolysis, hydration, cyclization and isomerization. The widespread and frequent use of manmade xenobiotic chemicals has led to a remarkable effort to implement new technologies to reduce or eliminate these contaminants from the environment. One promising treatment method is to exploit the ability of microorganisms to remove pollutants from contaminated sites. An alternative treatment strategy that is effective, minimally hazardous, economical, and versatile and eco-friendly is the process known as bioremediation. The present work was undertaken with the objective of obtaining indigenous bacterial strain having potential to degrade dichlorvas insecticide.

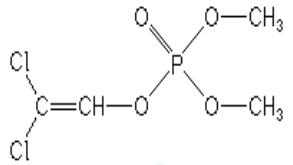


Fig.1: Structure of Dichlorvas

2. MATERIAL AND METHODS

2.1. Insecticide

The technical grade insecticide- dichlorvas(2,2-dichlorovinyl dimethyl phosphate)(76% E.C.) used in the present study was obtained from local market of Sangli, M.S. India.

2.2. Soil samples

The soil samples were collected from the grape wine yards of different locations of Sangli district, M.S. India, having known history of dichlorvas use since at least last five years.

2.3. Enrichment of dichlorvas degrading bacteria

The samples were enriched by adding 1 gm of soil into the mineral salts medium (MSM) containing 0.3gm $NaNO_3$, 0.05gm $MgSO_4$, 0.001gm $FeSo_4$, 0.1gm K_2HPO_4, 0.05gm KCl, 0.5gm KH_2PO_4, 0.05gm Yeast Extract and 1.0gm glucose, 100ml D/W, pH 7.0-7.2 and supplemented with 5,10, 15 and 20 mg /L of dichlorvas and incubated at 25-30°C for 7 days on orbital shaker at 200 rpm.

2.4. Isolation, selection and identification of dichlorvas degrading bacteria

An enriched culture was streaked on MSM agar plates added with same concentration of insecticide and incubated at 37°C for 24-48 hrs. Individual colonies were subcultured on MSM agar plates containing same concentrations of the dichlorvas until pure culture was obtained. The isolates showing highest degree of tolerance were maintained on agar slant at 4°C and subcultured after every two months. They were further processed for their morphological, cultural, biochemical and molecular characterization for their identification.

2.5. Identification of bacteria by using 16S rDNA based molecular technique

Amplification of 16s rRNA fragment was done from the genomic DNA of respective isolates for the analysis of phylogenetic relationship. 16s rRNA gene amplification was done by polymerase chain reaction and consensus sequence was generated by Aligner software. BLAST (Basic Local Alignment Search Tool) was performed with nr database of NCBI (National Center for Biotechnology Information)genbank by using sequence of 16S rRNA gene. First ten sequences were selected depending upon maximum identity score. Clustal W, a multiple alignment software program was used for alignment. Distance matrix was generated by using RDP database (Ribosomal *Database* Project) and phylogenetic tree was constructed by using MEGA 4 (Molecular Evolutionary Genetics Analysis).

2.6. Biodegradation of dichlorvas by selected bacterial strain

To study the degradation of dichlorvas, the selected isolate was inoculated in the MSM broth with 15mg/L concentration of dichlorvas and kept on rotary shaker at 150 rpm for incubation at room temperature for 8 days. The degradation was determined after every 2 days by measuring λmax of the compound at 280 nm by UV-visible spectrophotometer. For this, 5 ml of sample was collected after every two days of incubation and centrifuged at 10,000 rpm for 12 minutes in cooling centrifuge adjusted to 4°C.The pellet was discarded and supernatant was analyzed. The degradation activity was expressed as percent degradation which wasdetermined by following formula,

Percent degradation = Ab-Aa / Ab x 100
where,
Aa = Absorbance of compound after degradation at 220 nm
Ab = Absorbance of compound before degradation at the same wavelength.

2.7. Extraction of metabolites and GCMS analysis

The degraded broth was centrifuged at 10000 rpm for 15 min after 8 days of incubation. The supernatant was taken for extraction of metabolites with ethyl acetate (1:1). Evaporation of extracts was done in evaporator till dryness. The obtained residue was dissolved in small volume of methanol and used for GCMS analysis. The GCMS was performed in a specific temperature programming mode with a DB5MS 30m capillary column (0.25 mm column ID and 2.5 micron particle size). The sample was injected into temperature program with a split mode of 180°C for 1.5 min, 250°C for 10 min, at the normal rate of 10°C /min, injector temperature was 250°C and temperature of detector was 250°C. Carrier gas used was Nitrogen. Components were analyzed and compared with NIST (National Institute of Standards and Technology) library.

2.8. Effect of physicochemical parameters:

2.8.1. Effect of pH and Temperature

The effect of physicochemical parameters like pH and temperature were studied for the observation of

maximum degradation of the dichlorvas. Effect of temperature was studied by keeping media in illuminating incubators adjusted at 10, 20, 25, 30, 35, and 40°C respectively. To determine the effect of pH , media with pH values from 4.0 to 11.0 were prepared and all the optimal conditions were kept constant except pH and the variation in percentage degradation with respect to change in pH was studied.

3. RESULTS AND DISCUSSION

3.1. Selection and identification of dichlorvas degrading bacteria

A total of 13 bacterial isolates were obtained from collected soil samples. The code numbers given to these isolates and their tolerance limit to the dichlorvas is given in the Table 1. Among these isolates, three isolates namely WL DumpD20, MJ D20 and TS D20 were tolerating highest concentration (20 mg/L) of dichlorvas while BS D15,PL D15 and MJ D15 were tolerating 15 mg / L concentration of dichlorvas.

Table 1: Tolerance of isolated bacterial strains towards dichlorvas

Isolate Code	Bacterial growth on MSM containing Dichlorvas as carbon and energy source (mg/L)			
	5	10	15	20
WL DumpD20	+	+	+	+
WL DumpD10	+	+	–	–
BS D10	+	+	–	–
BS D5	+	+	–	–
BS D15	+	+	+	–
PL D10	+	+	–	–
PL D15	+	+	+	–
VT D10	+	+	–	–
MJ D15	+	+	+	–
MJ D20	+	+	+	+
TK D10	+	+	–	–
TS D20	+	+	+	+
TS D10	+	+	–	–

Note: '+' → *Growth* '-' → *No growth*

These six isolates were processed for their morphological, cultural, biochemical and molecular characterization and identification. However, the dichlorvas degradation potential of the isolate PL D15 is detailed here. This isolate was producing small, pale yellow, round and slimy colonies. It was gram negative, small rod shaped, nonmotile, non spore former, fermenting glucose and lactose but no sucrose and mannitol. It was positive for amylase, catalase, oxidase, H_2S, nitrate reductase and urease while it was negative for gelatinase.

The 16S rRNA sequence analysis showed its greatest sequence similarity with *Pseudochrobactrum saccharolyticum* ALK635 (Accession Number KC456600). Therefore, the isolate PL D15was identified as a strain of *Pseudochrobactrum saccharolyticum* (Fig. 2). The 16s rRNA sequence was submitted to the NCBI gene bank under the Accession Number KT427393.This bacterium had never been reported before for organophosphates degradation.

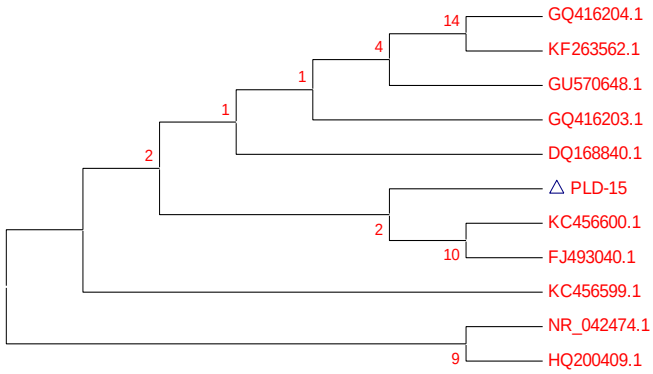


Fig. 2: Phylogenetic Tree of PL D15

3.2. UV-Vis analysis

Spectra were obtained at 100-400nm wavelengths by using UV-Visible analysis of cell free broth to confirm the degradation of dichlorvas by the strain of *Pseudochrobactrum saccharolyticum*. The UV-Visible spectrum of dichlorvas insecticide before and after degradation is shown in Fig. 3.

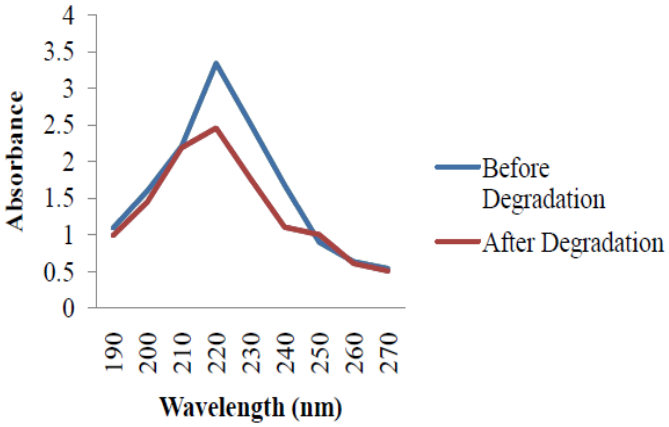
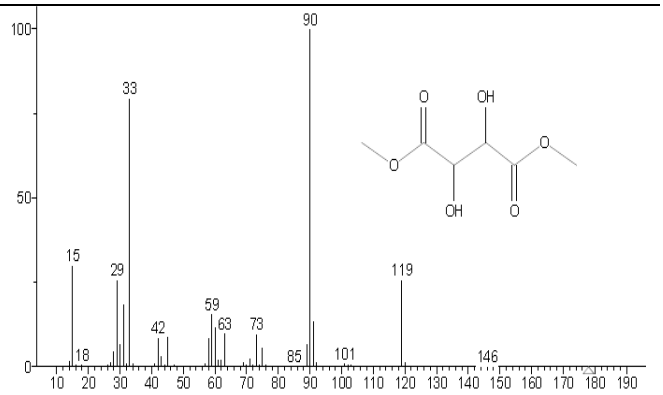
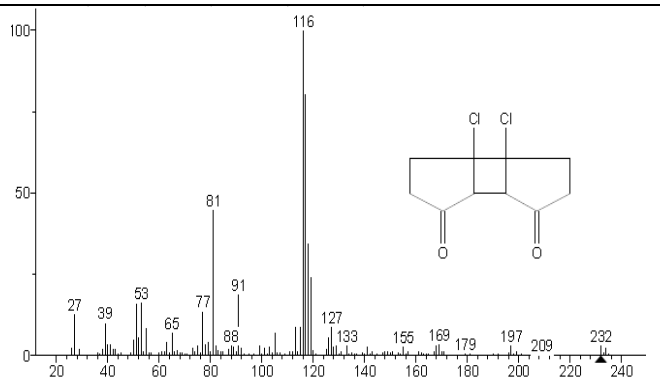
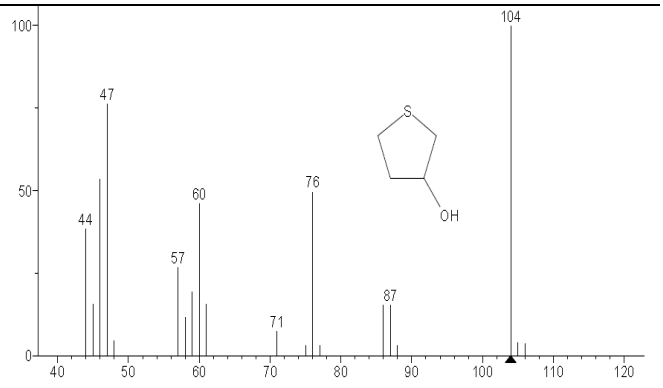


Fig. 3: Spectra of dichlorvas insecticide by using UV-Vis analysis before and after degradation by strain of *Pseudochrobactrum saccharolyticum* after 8 days of incubation

3.3. GCMS analysis

The metabolites of degradation by the strain were identified by GC/MS analysis using NIST library. Upon GC/MS analysis the dichlorvas shows the retention time of 7.208 min. The GC/MS of metabolites indicated the formation of 3 metabolites as butanedioic acid, 2, 3-dihydroxy-dimethyl-ester; tricyclo (5.3.0.0(2,6)deca-3,10-dione, 6,7-dichloro;and thiacyclopentane-3-ol. (Table 2).

Table No.2: GCMS analysis of extracted residues of Dichlorvas

Weight of metabolite (m/z)	Retention time (min)	Name of metabolite	Mass peak
178	5.853	Butanedioic acid, 2,3-dihydroxy- [R-(R*,R*)]-, dimethyl ester	
232	9.885	Tricyclo[5.3.0.0(2,6)] deca-3,10-dione, 6,7-dichloro-	
104	7.208	Thiacyclopentane-3-ol	

3.4. Effect of physicochemical parameters on Dichlorvas degradation

The effect of physicochemical parameters on the dichlorvas degradation by the strain was studied and observed that the highest degradation rate is at the temperature of 30°C (89.99%) and pH 7(90.12%) (Fig. 4 and 5).

The biodegradation of organophosphates by several bacteria such as *Pseudomonas aeruginosa , P. putida , Bacillus pumilus, Bacillus cereus, Serratiamarcescens, Klebsiella* sp.,

Alcaligenes sp., *Alcaligenes faecalis* , *Flavobacterium* sp., *Enterobacter* strain B14, *Agrobacterium* sp., *Arthrobacter* sp., *Stenotrophomonas* sp., *Ralstonia* sp., *Sphingomonas* sp. Dsp-2 has been already reported by several researchers [2].

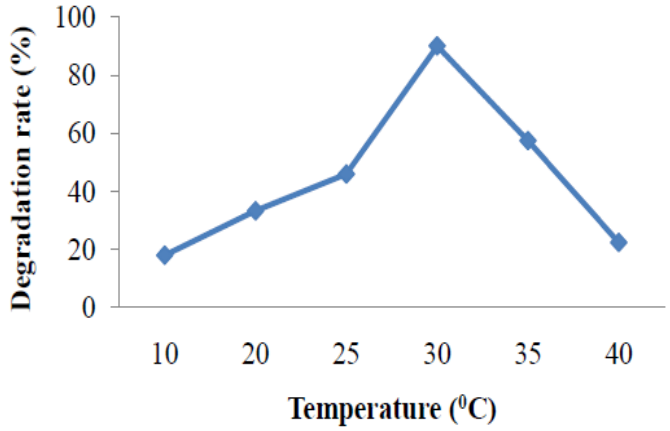


Fig. 4: Effect of temperature on degradation of dichlorvas

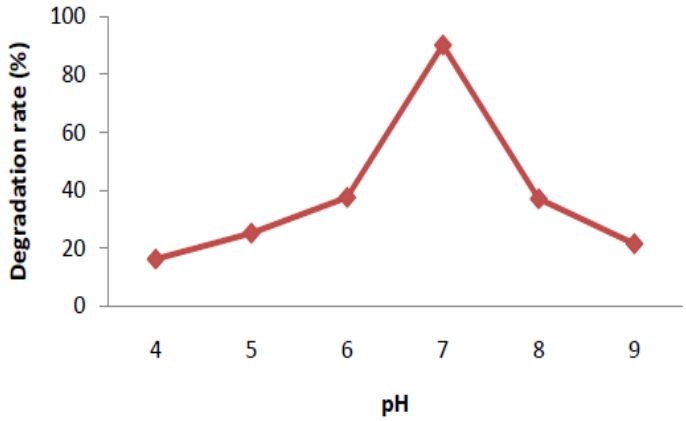


Fig. 5: Effect of pH on degradation of dichlorvas

The biodegradation of dichlorvas by the *Bacillus strain* WL Dump D10 has been previously reported where the metabolites of degradation by this strain were Pyridine, 3-ethenyl-; 5-Phenyl-1H-tetrazole and 2-Chlorohistidine [3]. It is also found that a strain YD4 of *Flavobacterium sp*. from rape phyllosphere having ability to degrade the dichlorvas and gave a new way for in situ phyllosphere bioremediation by the use of epiphytic bacterium [4].This investigation shows that the strain of *Pseudochrobactrum saccharolyticum* has ability to degrade dichlorvas into butanedioic acid, 2, 3-dihydroxy-dimethyl-ester; tricyclo (5.3.0.0(2,6)deca-3,10-dione, 6,7-dichloro;and thiacyclopentane-3-ol as identified by GCMS analysis. However, further research will be needed to clarify the degradation pathway and the properties of the key enzymes involved in its biodegradation.

Adichlorvos degrading strain DDV-1 of *Ochrobactrum sp.* was isolated and determined its effectiveness in the bioremediation of a dichlorvas contaminated soil (5).The strain DDV-1 was able to utilize dichlorvas as a sole carbon source, and the optimal pH and temperature for its cell growth and degradation were 7.0 and 30°C, respectively. *Pseudochrobactrum* sp. XF1-UV could be a promising candidate for bioremediation of phenol containing wastewater or for bioremediation of phenol contaminated sites [6].

Thus, the newly isolated strain of *Pseudochrobactrum saccharolyticum* has potential to degrade dichlorvas. After elucidating the degradation pathway and the properties of the key enzymes involved in its biodegradation, it could be used as a promising organism for the bioremediation of grape wine yard as well as other soils that are polluted with the excessive use of dichlorvas.

4. REFERENCES

1. Parsek MR, McFall SM, Chakarbarty AM. *Biosorption and bioremediation,* 1995; **35**:175-188.
2. Dhanya MS. *Journal of Environmental Research and Development,* 2014; **9(1):**232-240.
3. Pawar KR, Mali GV. *International Research Journal of Environment Sciences,* 2014; **3(5):**8-12.
4. Jiying N, Gang G, Zhihui B, Qing HU, Hongyan QI, Anzhou MA, XuliangZ, Guoqiang Z. *Front. Envir. Sci. Eng.*, 2012; **6(2)**:231-237.
5. ZhangXH, Zhang GS, Zhang ZH, JianHong XU, ShunPeng LI. *Pedosphere*, 2006 ;**16(1)**: 64-71
6. Zhen M,Chenyang Y, Lingling X. *Int. J. Mol. Sci.*, 2015; **16(4)**:7320-7333.