STUDIES ON ISOLATION, CHARACTERIZATION AND GROWTH OF CHLORPYRIFOS DEGRADING BACTERIA FROM FARM SOIL

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Abstract:

In the present work, three bacterial strains were isolated from pesticide decipitated chilli crop field soil, Dharwad district, state Karnataka, possessing the degrading capacity of Chlorpyrifos. Identification of bacterial strain was carried out by biochemical tests. The bacterial strains were inoculated in a nutrient media containing Chlorpyrifos, as a carbon source 200mg/l and incubated at 30^{o}C for 24 hours in a rotary shaker. All strains were identified by biochemical tests, and named them as DSB1, DSB2 and DSB3. Further the degradation of Chlorpyrifos by the three strains was analysed by high performance liquid chromatography and the degradation by the three strains was compared. The strain DSB1 showed significant degradation ability in comparison with DSB2 and DSB3 strains, degrading 71.2% of total Chlorpyrifos.

***Keywords**: Bacteria, Chlorpyrifos, Characterization, Degradation, Growth response Isolation.*

1. INTRODUCTION:

Pesticides are xenobiotic compounds used worldwide to prevent the crops, forestry and horticulture damages from insect, fungi, pests and other diseases, to adjust the growth of the plants or to increase the yields of the crops (4,5).

The degradiation of organophosphrous compounds in the atmosphere has been studied extensively. The mode of action of these is direct attack on the nervous system by inhibiting acetylcholinesterase synthesis. Due to this they are linked to neurotoxic compounds. Chlrophyrifos (*O*,*O*-diethyl *O*-3,5,6-trichloropyridin-2-yl phosphorothioate) is an organophosphate insecticide used majorly in the field of agriculture (1). The use of this insecticide has increased in recent years because it is less toxic than other organophosphrous compounds such as Methyl parathion, Methamidophos etc. (6). The half-life of this fungicide varies from 10-120 days based on the variety of soil, variation in abiotic and abiotic factors. Where it degrade to 3,5,6-trichloro-2-pyridinol (TCP) (2). The earlier research has been reported the antimicrobial properties of TCP in soil which inhibit the growth and proliferation of the soil micro flora and bring down the degradation process (3), leading to accumulation of residues of Chlorpyrifos in soil and environment. Therefor the present study was focussed on the isolation of Chlorpyrifos degrading bacteria from the Chlorpyrifos applied soil and their ability to degrade this insecticide.

2. MATERIALS AND METHOD:

2.1. SOIL SAMPLE COLLECTION, CHEMICALS AND MEDIA PREPARATION:

The soil samples were collected from agricultural university Dharwad from the chilli fields with the history of 5 years application of Chlorpyrifos. The commercial grade of Chlorpyrifos was purchased from the local pesticide suppliers, Dharwad. The other analytical grade chemicals and reagents were procured from Himedia. Nutrient agar media with the following composition Peptic digest of animal tissue 5g, Sodium chloride 5g, Beef extract 1.5g, Yeast extract 1.5g and Agar 15g /ltr and pH 7.4±0.2. used by autoclaving at 121^0C temperature, 15 psi pressure for 20 minutes

2.2. ISOLATION AND PURIFICATION OF BACTERIA DEGRADING CHLORPYRIFOS:

1g of soil samples obtained from the fields dissolved in 9ml saline and carried for serial dilution. After serial dilution the samples (100µl) were plated on nutrient agar media. All plates were incubated at 37^0C, for 24 hours in an inverted position. Each single colony obtained was streaked on nutrient media to obtain the pure culture. Each isolated colony is then streaked on nutrient media amended with 0.02% of Chlorpyrifos to obtain the Chlorpyrifos resistant/ degrading bacteria, incubated at 37^0C. For 24-48 hours. Each resistant colony was streaked again Chlorpyrifos containing media for the resistant bacterial isolates. Each isolated single stain is then inoculated in 100ml of nutrient broth and incubated at 37^0C for 24 hours, used for further characterization. Each isolate was refrigerated to retain the viability.

2.3.CHARACTERIZATION AND IDENTIFICATION OF BACTERIA:

The study of colony morphology was done by streaking single isolated colony on nutrient agar media. The morphological features such as size, shape, texture, opacity, colour etc. was studied by direct observation. The gram nature of each bacterial strain was studied by gram staining. Further each strain was incorporated to biochemical test for characterization such as

motility, catalase, oxidase, starch hydrolysis, coagulase, urease, methyl red, citrate utilization, nitrate reduction, Voges-Proskauer, Indole, H2S production, Gelatin hydrolysis etc. were carried as per Cheesbrough(1993) and Benson (1994).

2.3 . DEGRADATION STUDY BY BACTERIA:

The growth pattern was studied for screened bacterial strains by optimising temperature, pH of the medium. For optimisation of these two important parameters, the bacterial strains were inoculated on a 150ml sterilized nutrient medium amended with 0.2% Chlorpyrifos. For optimising temperature and pH, all inoculated strains were incubated at five different temperature viz. 20 ^{0}C, 25^{0}C, 30^{0}C, 37^{0}C and 48^{0}C. and pH set at 5,6,7,8,9 set before sterilizing the medium. After incubation for 48 hours, at 600nm the absorbance was taken. Whole experiment was done in triplicates and the mean value was used to plot the graph for optimising temperature and pH for screened bacterial isolates. Non inoculated medium was served as control.

2.4 . QUANTIFICATION OF DEGRADATION BY HPLC:

Chlorpyrofos degradation by soil isolates was quantified by High profile liquid chromatography with different incubation time. MSM media was prepared with 0.2% chloropyrifos as a sole carbon source. Degradation levels of Chlorpyrifos assayed by HPLC (Tamimi*et al.*, 2006) on a Shimadzu HPLC System using a C-18 (150mm X 3m, 3µm), the flow rate of 1ml per minute and the injection volume was 10µl. the isocratic elution conditions were methanol and water (70:30 v/v),the wavelength for detection was 230nm. The control batch was non inoculated sample containing Chlorpyrifos in 100ml of nutrient medium. In the test sample 5ml culture was added to the sterile nutrient growth containing Chlorpyrifos. Incubated in a rotary shaker at 150rpm at 37^{o}C and the percentage of degradation were analysed at 24, 48, 72 and 96 hours.

3. RESULTS:

3.1. ISOLATION, IDENTIFICATION AND STRAIN CHARACTERIZATION.

Isolation of bacteria was done on nutrient media by soil dilution method. Further the screenings of Chlorpyrifos degrading bacteria was done by amending the minimal salt media with 0.2% Chlorpyrifos as a sole carbon source and incubated at 37°C for 24-48 hours and were observed to study the colony morphology. Further the colony characteristics were studied by standard biochemical tests.
On the basis of morphological, gram nature and biochemical properties, the isolated bacterial strains were identified as Bacillus sp.(DSB1), Klebsiella sp.(DSB2) and Pseudomonas sp.(DSB3) respectively. The morphological and biochemical properties were presented in table (1,2).

3.2. EFFECT OF TEMPERATURE ON BACTERIAL GROWTH:

As per the experimental observations the maximum growth was observed between 20°C to 45^{0}C. at low temperature the growth was comparatively low and high temperature the growth and activity has reduced. DSB1 strain was shown good activity and growth at temperature 30°C -37°C. DSB2 was active at 37°C. DSB3 strain had shown good growth even at high temperature that is above 37°C .Therefore the optimisation was required to obtain proper

growth and activity of Chlorpyrifos degrading bacteria. From the above observations 37°C was set for the best growth and activity of all three isolates, presented in (graph 1) .

3.3. EFFECT PH ON THE GROWTH OF BACTERIA:

The PH of the soil has great influence on bacterial population as well as degradation of synthetic compounds. The optimum growth pH was 6.5-7.5 respectively. In all cases the maximum growth was observed at 7.0, with the increasing in pH there was a sharp decrease in the growth of the bacterial isolates. Therefore the optimisation of pH of the medium holds a great influence on bacterial growth and activity, presented in (graph 2).

3.4. QUANTIFICATION OF DEGRADATION BY HPLC:

All strains were shown the degrading capability of Chlorpyrifos. The strain DSB1 was more potent by degrading the 72.38% of total compound from the media at 96 hours of incubation. The other two strains DSB2and DSB3 showed 37% and 57.98% of degradation respectively, presented in (graph 3).

4. CONCLUSION:

The current study included the isolation, characterization of efficient soil bacterial isolates for the degradation of Chlorpyrifos. The pH and temperature showed their on bacterial growth and their activity. The mineral salt medium supplemented with 0.02% compound as a carbon source at 37°C, pH 7 showed 72.38% of degradation by DSB1 strain. The study indicated that the pesticide contaminated soil can provide ample number of micro flora capable of degrading various form of pesticide over the period, as these microbes quickly adapt to the provided environmental conditions and can be utilized further for genetic medication for better yield and can be used for bioremediation and biodegradation.

5. ACKNOWLEDGEMENT:

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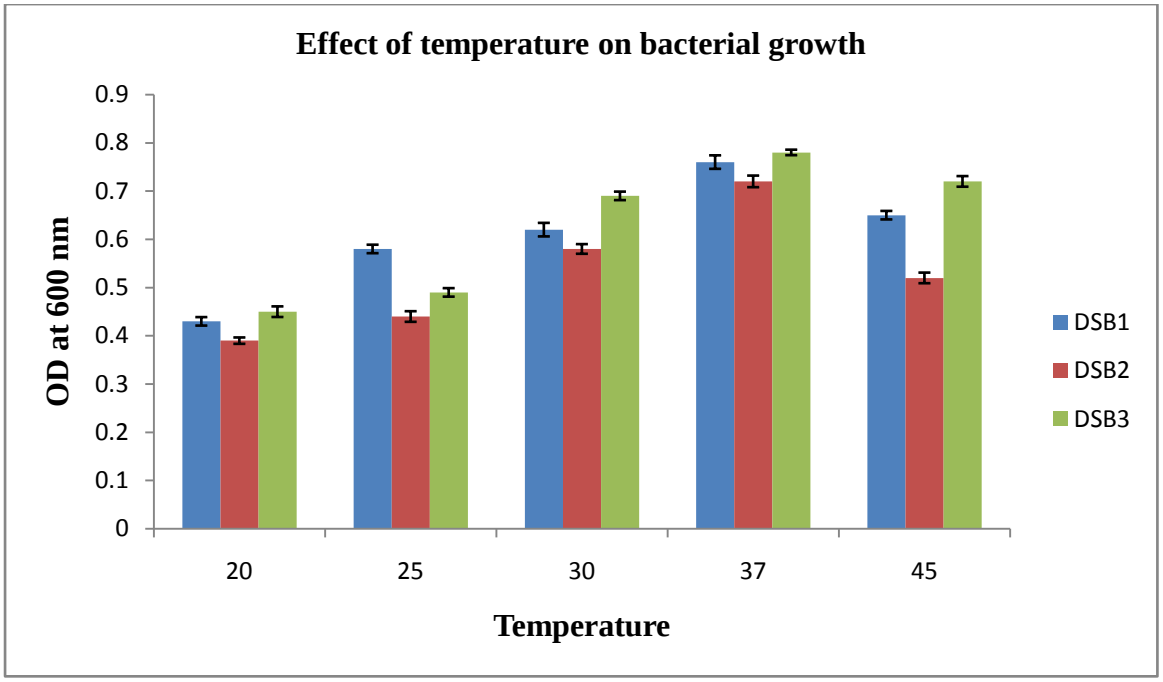
Table 1. Morphological characteristics of the soil isolates

Isolate no.	Margin	Surface	Elevation	Opacity	Colour	Consistency	Gram's nature
1	DSB1	Smooth	Convex	Opaque	White waxy	Smooth	Gram +ve
2	DSB2	Smooth	Convex	Translucent	Slimy white	Smooth	Gram - ve
3	DSB3	Smooth	Convex	Translucent	Bluish-green	Smooth	Gram – ve

Table 2.Biochemical characteristics of isolated strains.

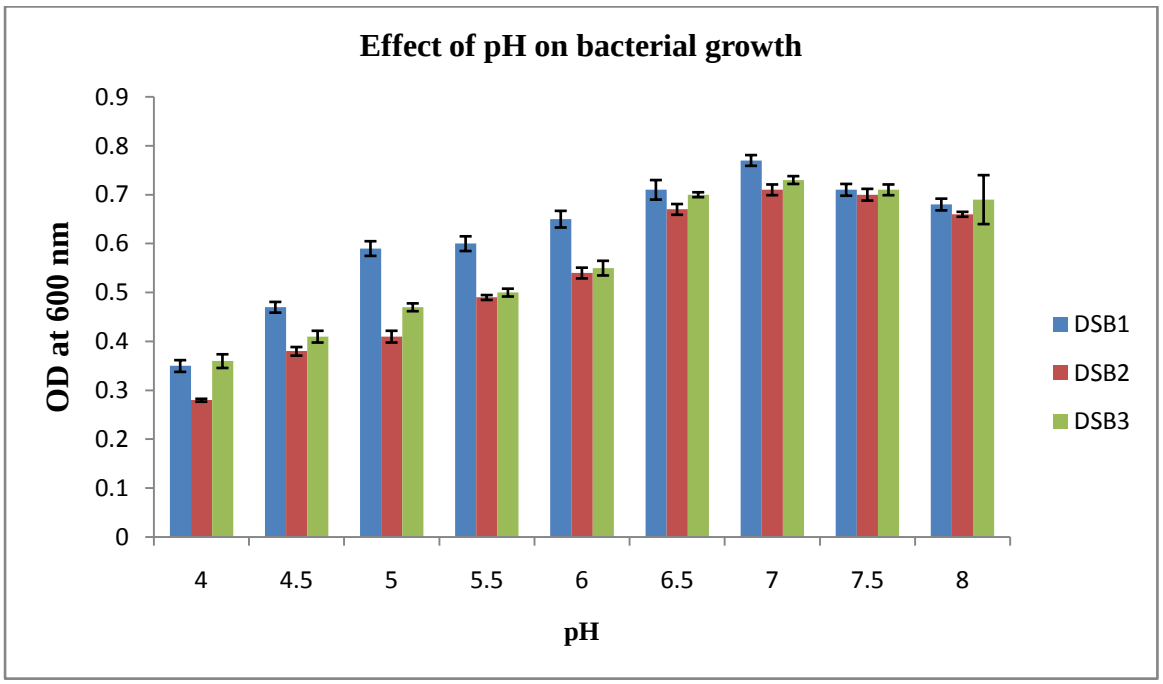
Tests	Soil isolates		
	DSB1	**DSB2**	**DSB3**
Gram Stain	**+ve**	**-ve**	**-ve**
Agar plate morphological characteristics	Abundant, opaque, white waxy growth	Slimy, White, somewhat Translucent, raised growth	Abundant, thin, white medium turns green
Gelatinliquification	+	-	+
Starch hydrolysis	+	-	-
Lactose	-	AG	-
Dextrose	A	AG	-
Sucrose	A	AG	-
H_2S Production	-	-	-
NO_3 Reduction	+	+	+
Indole Production	-	-	-
MR Reaction	-	-	-
VP Reaction	+ -	+ -	-
Citrate Utilization	-	+	+
Urease Activity	-	+	-
Catalase Activity	-	+	+
Oxidase Activity	+	-	+

Graph 1: effect of temperature on bacterial growth and activity



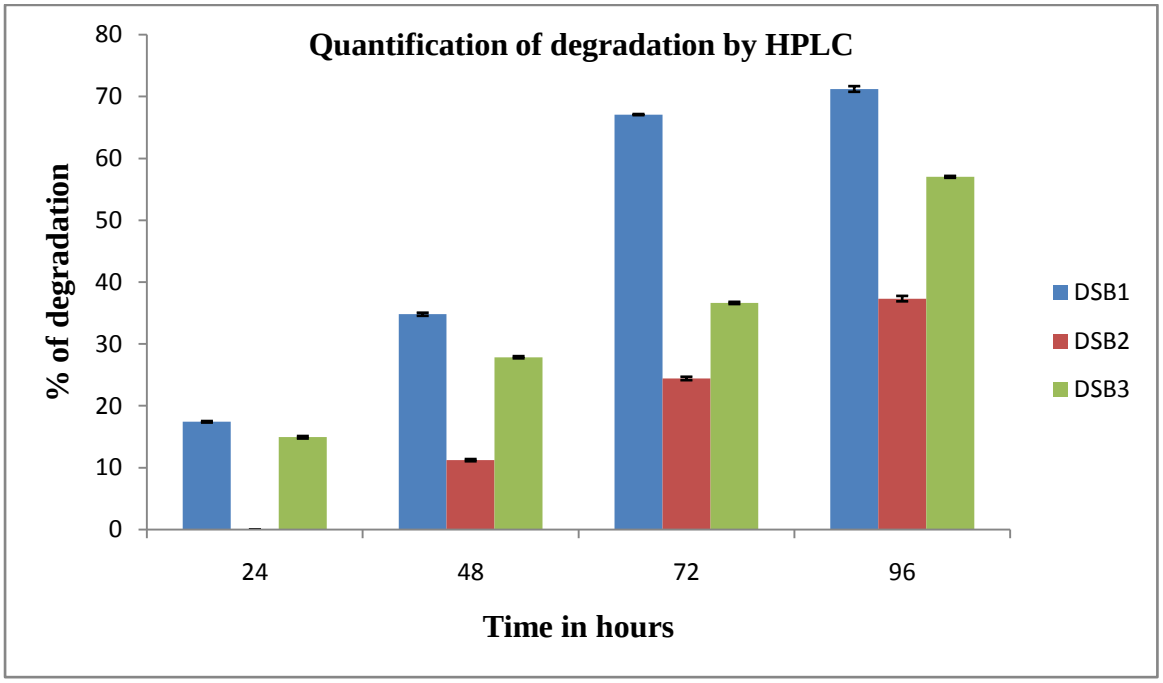
Note: Significance P≤0.5

Graph 2: effect of pH on bacterial growth and activity



Note: Significance P≤0.5

Graph 3: Quantification of degradation by HPLC



Note: Significance P≤0.5

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