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Screening, Isolation and Characterization of Cypermethrin Tolerating Bacteria from Sugarcane Field Soil

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Abstract

Cypermethrin is a synthetic pyrethroid pesticide group widely used to control pests in cotton, fruits, and vegetable crops. Excessive use of pesticide in agriculture is one of the major causes of environmental pollution. The present study was aimed to screen, optimize and identify Cypermethrin-tolerant bacteria. For this purpose the pesticide exposed soil samples were collected and bacterial strains were isolated on Cypermethrin-containing Nutrient agar plates. On the basis of physical and chemical characterization, ten isolates were obtained named as KCl to KC10. The isolate KC1 was grown in Nutrient Broth Medium (NBM) at various pH, temperature, NaCl concentrations, carbon source and nitrogen source. The optimum growth pH and temperature of KC1 were found to be pH 7 and 37 °C, respectively. About 3% NaCl concentration was found to be optimum for growth. Optimum growth was obtained while using glucose as a carbon source and beef extract as a nitrogen source.

Keywords: *Bacillus, Cypermethrin, degradation, KC 1, pyrethroid, soil*

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INTRODUCTION

Pyrethroids are synthetic compounds analogous to pyrethrins, which are derived from *Chrysanthemum cinerariaefolium* plant. Cypermethrin is a synthetic pyrethroid pesticide group widely used to control pests in cotton, fruits, and vegetable crops [1**]**. The use of Cypermethrin has been increased in agricultural field to protect crop from various pests. Only about 0.1% of applied pesticides reaches the target organism while the remaining bulk pollutes the soil and nearby environment [2**]**. With the growing use of cypermethrin in agriculture, its impact on the soil microflora and other environmental parts has received more attention [2–4**]**. Toxic effects of Cypermethrin have been described in various categories, e.g. genotoxic, neurotoxic, immunotoxic and carcinogenic to mammals, including humans by many researchers [5–7].

Microorganisms express significant capacity for the metabolism of pesticides. Although they are capable of catalyzing similar metabolic reactions as mammals and plants, they own the unique ability to mineralize various xenobiotics [8]. Hence, biodegradation, ideally the target pesticides will be able to serve as the carbon source and energy for the microorganisms. The surviving bacteria under pesticides stress can provide an efficient, cheaper and ecofriendly solution for bioremediation of the pesticide-contaminated soil [9**]**. Therefore we aimed to screen, isolate and identify the Cypermethrin tolerating bacteria from the cultivated soil for further biodegradation study.

MATERIALS AND METHOD

Sample Collection

The soil sample used in the present study was collected at 10 cm depth from the agricultural field from Bardoli Taluka, Gujarat, India (21° 7' 29.4852" N; 73° 6' 45.3960" E) having pesticide application history. The soil sample was collected in a sterile polythene bag and stored at 4°C until further use.

Enrichment and Isolation of Soil Microorganisms

Enrichment culture technique using Nutrient Broth Medium (NBM) supplemented with pesticide as sole carbon and energy source was

used for isolation of pesticide degrading bacteria. Briefly, about 10 g of soil was added to a 250 ml Erlenmeyer flask containing 100 ml sterile NBM supplemented with 0.2% v/v Cypermethrin and incubated at 30°C under shaking condition for a week. After a week, 10 ml of this culture was transferred to 100 ml of fresh sterile NBM containing 0.4% v/v Cypermethrin and incubated under the same conditions. Likewise, the concentration of pesticide was gradually increased from 0.2% v/v to 1% v/v on weekly basis with an increment of 0.2% v/v. At every incremental step the culture was serially diluted and spread plated on to the corresponding solid NA and incubated at 30°C for 2–4 days. The representative microorganisms growing on the plates were purified following the four-flame streaking method. The isolates withstanding highest pesticide concentration (1% v/v) were finally selected for further study.

Characterization of Various Parameters for Bacterial Growth

Effect of Temperature on Bacterial Growth

100 µl of the active bacterial culture was inoculated in 100ml NBM enriched with 1% Cypermethrin and incubated at different temperatures such as 27oC, 30o C, 37o C and 47o C. After 24 hours incubation, the growth was measured at every 24 hrs time interval by taking the optical density (OD) at 600 nm up to 96 hours. Uninoculated control served as blank and the growth curve was plotted.

Effect of pH on Bacterial Growth

Sterile NBM supplemented with 1 % v/v Cypermethrin having different pH values such as 5, 6, 7, 8 and 9 were prepared. The pH was adjusted with sodium hydroxide and hydrochloric acid solutions. 100 µl the bacterial culture was inoculated and incubated at 37o C for 24 hrs. The growth was measured at every 24 hrs by taking the optical density (OD) at 600 nm up to 96 hrs. Uninoculated control served as blank and the growth curve was plotted.

Effect of NaCl concentration on bacteria growth

100 µl of the active bacterial culture was inoculated in 100ml NBM enriched with Cypermethrin with different NaCl concentrations i.e. 1%, 3%, 5% and 9% w/v. Inoculated broth was incubated at 37oC for 24 hours. The growth was measured at every 24 hrs by taking the optical density (OD) at 600 nm up to 120 hrs. Uninoculated control served as blank and the growth curve was plotted.

Effect of Carbon source on bacterial growth

Sterile nutrient broth enriched with 1% v/v Cypermethrin prepared with different carbon sources like glucose, xylose, lactose, sucrose and fructose followed by inoculation of isolated strain. The growth was measured at every 24 hrs by taking the optical density (OD) at 600 nm up to 96 hrs. Uninoculated control served as blank and the growth curve was plotted.

Effect of Nitrogen source on bacterial growth

Sterile nutrient broth enriched with 1% v/v Cypermethrin was prepared with different Nitrogen sources like yeast extract, beef extract, NH_4NO_3, $NaNO_2$ and KNO_3 followed by inoculation of isolated strain. The growth was measured at every 24 hrs by taking the optical density (OD) at 600 nm up to 96 hrs. Uninoculated control served as blank and the growth curve was plotted.

RESULTS AND DISCUSSION

In the present study, pesticides tolerating ten different colonies (KC1 to KC 10) were isolated on Nutrient agar containing pesticide by enrichment method. From which most rapidly growing bacterial colony KC1 was selected for further study.

Figure 1 represents the effect of temperature on isolated bacterial growth. It shows a good growth was observed at 37 °C and is gradually decreasing with 30 °C, 45 °C and 25 °C. The pH is an important indicator of chemical changing of the soil. The studies indicated that survival bacteria cell in soil was strongly influenced by pH and organic matter. This strain could engage in efficiently tolerate Cypermethrin over a wide range of pH 6, 7, 8 and 9. The surprising results were noticed at pH 6 and 9 the bacterial growth remain negligible upto first 3 days. The organism shows higher growth at pH 6 & 7 (Figure 2). The isolated strain also tolerates higher concentrations of NaCl upto 4 days, while 1%

and 3% of NaCl were favourable to the strain to grow (Figure 3). It indicates that the organism can survive also in harsh conditions for some days. The strain utilizes glucose as a carbon source and shows maximum growth, poor growth observed in fructose followed by Lactose and sucrose shows least growth (Figure 4). In beef extract and yeast extract maximum growth recorded. The results indicated that in other nitrogen sources organism shows poor growth (Figure 5).

The result was similar with the report of Sankaralingam et al (2013), he reported that the Maribacter sp AMSU have been shown maximum growth at pH 7 [18]. Earlier it was reported that the bacterial isolates showed maximum growth in the presence of glucose [10].

Similar results were also reported in previous literature. Enterobacter strain B-14 used chlorpyrifos as a source of carbon and phosphorous [12]. Sethunathan and Yoshida (1973) isolated a Flavobacterium sp. that could use parathion as source of phosphorous but not diazinon as carbon source [13]. Bacillus amyloliquefaciens, Bacillus pseudomycoides and Bacillus licheniformis were also isolated and identified as Malathion tolerating bacteria [14]. Bacillus cereus ZH-3 and Streptomyces aureus HP-S-01were also used to degrade cypermethrin [15]. Micrococcus sp. was also isolated and identified as good cypermethrin degrading bacteria by Tallur et al., (2008) [6]. Other five pesticide tolerating bacterial strains and the tolerance property transferred to E. coli DH5α by plasmid DNA sample which shows tolerance property against pesticide were also isolated and identified [5]. Bipte et al., (2012) studied five different bacterial species which tolerate the higher concentration of pesticide and used for their bioremediation [17].

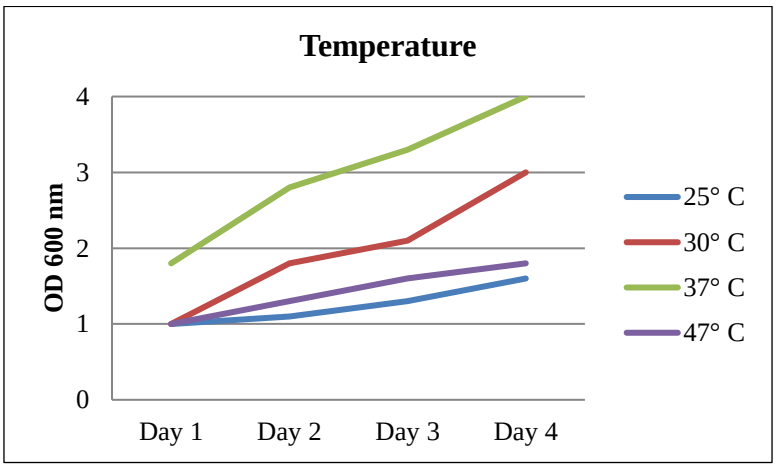

Fig. 1: *Effect of Temperature on KC1.*

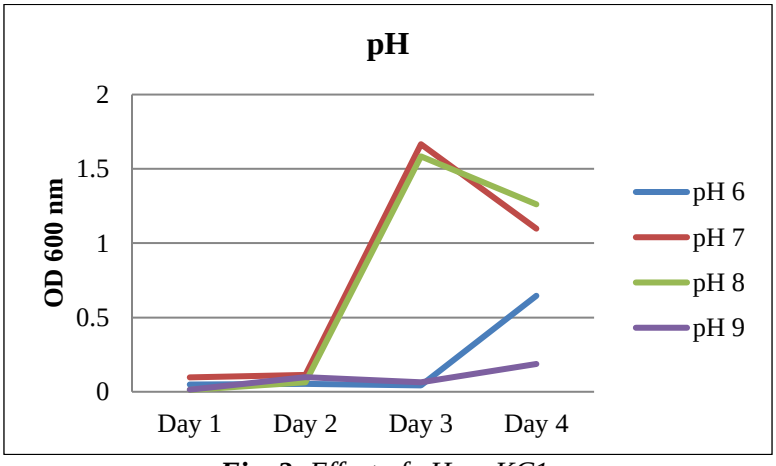

Fig. 2: *Effect of pH on KC1.*

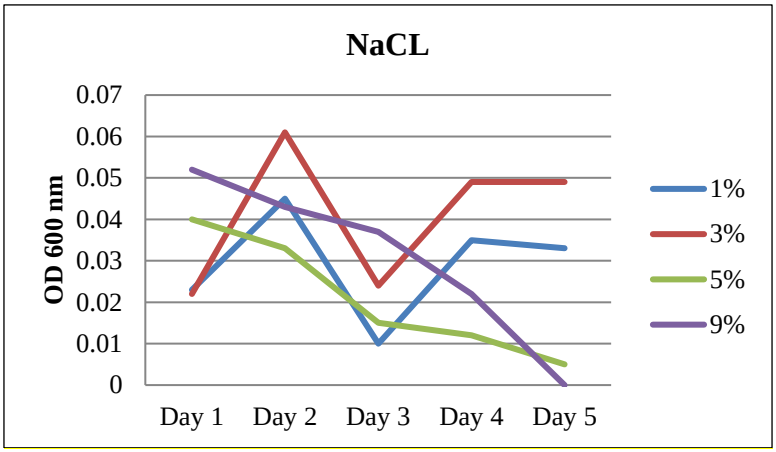

Fig. 3: *Effect of NaCl on KC1.*

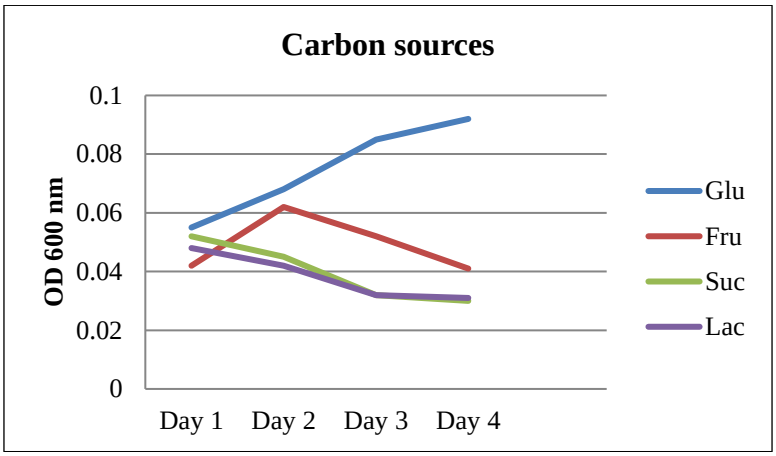

Fig. 4: *Effect of Carbon Source on KC1.*

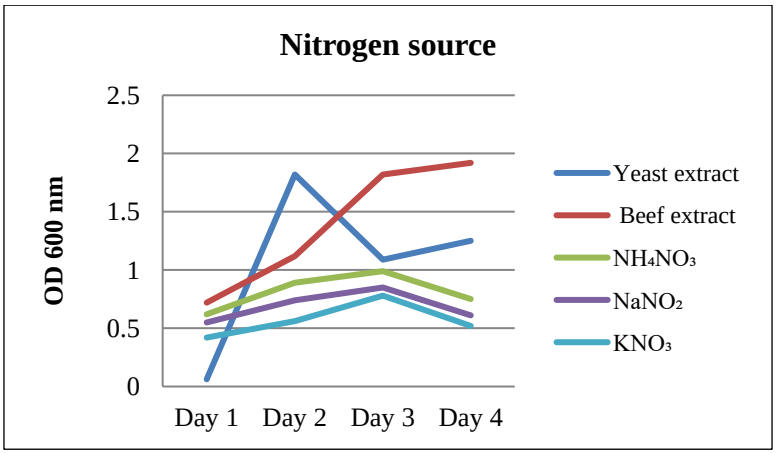

Fig. 5: *Effect of N_2 Source on KC1.*

CONCLUSION

Knowledge obtained from this study could help in understanding the optimum physicochemical parameters to culture KC1 which has a good tolerance capacity against Cypermethrin. This can be more essential for the researchers working in the field of pesticide biodegradation. In other words, it can be concluded that the isolated strain has capacity to tolerate and utilize Cypermethrin as a nutrient source. The media optimization results indicated that the isolated bacterial strain has efficiency to degrade Cypermethrin and effectively utilize it for bioremediation of the pesticide contamination.

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