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Pesticide Lambda-Cyhalothrin Degradation Using Mesorhizobium sp. (S₁b) and Bartonella sp. (S₂b) Strains Isolated from Cotton Crop

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Abstract

Lambda-cyhalothrin (LC), synthetic pyrethroid pesticide is used to control a wide range of pests in variety of agricultural fields. Pesticides are potentially harmful environmental pollutants and pose serious threat to human health. Very limited options are available for environment friendly removal of LC. Interestingly, soil microbes have been known to possess remarkable genetic makeup that helps them to perform vital job in cleaning-up harmful pollutants from the environment. In present study, two LC-degrading bacteria viz. *Mesorhizobium sp.* strain S₁B (Accession no. gb|MF471843|) and *Bartonella sp.* strain S₂B (Accession no. gb|MF471844|) were isolated by soil enrichment technique from cotton crop soil and characterized taxonomically using conventional methods and molecular PCR-based 16S rRNA sequence homology. The bacterial strains S₁B and S₂B achieved 29% and 40% removal of LC (conc. 250 mg/L, w/v), with maximum growth absorbance (OD) of 1.19 ± 0.06 and 1.13 ± 0.09 , respectively, during 20 days of incubation at 30°C and agitation 200 rpm under experimental laboratory circumstances. The percent removal of LC was estimated using UV-Vis Spectroscopy at 287 nm (λ max) against the standard curve plotted at different LC concentrations. The bacterial isolates of present study have exhibited substantial efficiency for environmental biodegradation of the pesticide.

Keywords: Lambda-cyhalothrin, Pesticide, Pyrethroid, Biodegradation, *Mesorhizobium sp., Bartonella sp.*

Introduction

The day by day increased use of pesticide in an agricultural field enhances the environmental pollution and hazards to the biodiversity [1]. Lambda-cyhalothrin (LC) is a synthetic pyrethroid insecticide, with its chemical name as (RS)-alphacyano-3-phenoxybenzyl3-(2-chloro 3,3,3 trifluoro-propenyl)-2,2, dimethyl cyclopropane carboxylate, introduced in 1988 [2]. Pyrethroid pesticides are highly active neurotoxic and have been mostly used in agricultural filed to control large number of

insects [3]. Although, a broad range of pests attacking several crops, i.e. cotton, cereals, hops, ornamentals and vegetables have been managed through application of proposed concentrations of LC [2]. However, it is highly lethal to organisms when discharged into natural environment. The LC is unusual amongst pyrethroids compounds and cause lethality by means of the external dermal exposure [2]. The chemical structure of LC is given in Fig. 1.

Figure 1. Chemical structure of Lambda-cyhalothrin

Environmental exposure of pesticides is a significant health risk for humans and other animals [4-5-6]. Pesticides not only cause many chronic irregularities in human beings but may also degrade biodiversity and overall environmental quality [7-8]. To address this life-threatening environmental issue, different physicochemical methods such as incineration, land filling, burning or composting, and chemical amendments have been used over the last few decades to remove pesticide pollution from the contaminated environments [9-10-11-12-13-14-15 and However, high cost and low efficiency often limit their use in remediation [14-15]. Pesticides are degraded by chemical and microbiological processes. Chemical degradation occurs through reactions such as photolysis, hydrolysis, oxidation and reduction [11]. Whereas biological degradation takes place when soil microbes utilize or break down pesticides because soil is the sink which receives the pesticides and it is the soil that uses microorganisms, which are responsible to degrade the compounds [10-12-13]. Bioremediation is innovative technology that has the potential to alleviate pesticide contamination. The process of bioremediation usually occurs in soil, whereby pesticides are broken down into less active/toxic compounds by fungi, bacteria, and other microorganisms that use pesticides as energy and carbon sources [16]. The decontamination and detoxification of the polluted environment are essential. A number of methods, including chemical treatment, volatilization, photodecomposition and incineration, can be applied for the detoxification of LC [17]. Bioremediation offers several advantages over the conventional chemical and physical treatment technologies, especially for diluted and widely spread contaminants [17]. Bioremediation, which involves the use of microbes to detoxify and degrade pollutants, has

received increased attention as an effective biotechnological approach to clean up polluted environments [18]. Studies of microbial degradation are useful for the development of bioremediation processes to detoxify pesticides [19]. Thus present study sought to explore the degradation of LC using indigenous Mesorhizobium sp. (S₁b) and Bartonella sp. (S₂b) strains isolated from cotton crop soil where LC was applied as pesticide.

Materials and Methods

The present study was carried out in Post-Graduate-Research Laboratory (PGRL), Institute of Microbiology, Shah Abdul Latif University, Khairpur, Sindh, Pakistan. Some of the analytical studies were also performed collaboratively at Research Microbiology Laboratory Department of Microbiology, Faculty of Biological Science, Quaid-i-Azam University, Islamabad, Pakistan. All the values reported in this manuscript average readings replicated represent of experiments unless mentioned otherwise.

Samples collection

Pesticide polluted soil samples (n=02) were collected after three days of pesticide fumigation from cotton crop fields in a depth of approximately 20 mm using agricultural knife. Samples were collected aseptically in sterilized polyethylene bags from two different fields of Taluka Kingri, District Khairpur Mir's, (1) Kacha Territory (K.T) Pir Sahib and (2) K.T Lalan Shah. The collected samples were labeled (date, day, time, sample type, and quantity) and transported to the PGRL where stored at 4° C until inoculation.

Isolation of LC-degrading bacteria

The soil samples collected from both sites were suspended into sterile distilled water (1 gram per 100 ml) each in 250 ml conical flask. The soil suspension was then mixed and kept at agitation in the environmental shaker (100 rpm, 30 minutes). Afterwards, this suspension was left at room temperature for 30 minutes to allow the settling of soil particles. The shaking flask containing 49.9 ml Mineral salts medium (MSM) broth was then

separately inoculated with the 0.1ml sample to make total volume of 50ml for the isolation of bacteria. The medium containing (in gram per liter); Na₂HPO₄ $2.0 \, \text{g}$, $\text{KH}_2\text{PO}_4 \, 0.75 \, \text{g}$, MgSO₄.7H₂O 0.5 g, NH₄ Cl 1.0 g, was used for the isolation of bacterial strain according to previously reported method [20]. The mineral salts medium was sterilized separately by autoclaving at 121°C for 15 minutes under 15lbs pressure. The pesticide (LC) was filter sterilized using an sterile membrane filter (Pore Size, 0.22 µm. Millex GP., Millipore, Bedford, Mass) and added aseptically to the medium at a final concentration of 250 mg/L (w/v). The pH of that medium was adjusted to 7.0 ± 0.2 using 6 N HCl and / or 1 M NaOH solution and used for isolation of LC-degrading bacteria by shaking flask method. All inoculated flasks were incubated at 30°C temperature for 20 days at 200 rpm in Multi-tier environmental shaker (Innova 4900, Germany). The experiments were performed in triplicate for both samples including positive and negative controls. The samples yielding maximum optical density (OD) were selected for the isolation of bacteria. The selected samples were streaked on the surface of MSM agar (MSM added with 1.5 % Agar-agar) plates and incubated at 37°C for 24 to 72 h. After incubation, the bacterial cultures were observed for their morphology and the bacterial colonies showing dissimilar features were purified and grown on nutrient agar medium for further identification process.

Identification of LC-degrading bacteria

The selected pure bacterial isolates showing LC-degradation were identified using microbiological taxonomic followed by the molecular confirmation using 16S rRNA gene sequence homology carried out commercially at Macrogen Inc., Seol, Korea. Consequently, 16S rRNA gene was amplified using set of forward and reverse universal primers, i.e. 27F (5'-AGAGTTTGATCMTGGCTCAG-3') and 1492R (5'-TACGGYTACCTTGTTACGAC TT-3'), respectively. Later, the amplified 16S rRNA gene was sequenced using universal 518F (5'-CCAGCAGCCGCGGTAATACG-3') (5'-TACCAGGGTATCTAATCC-3') primers. Finally, the gene sequences of approx.

1400bp were retrieved and studied for their sequence homology using bioinformatics tools like basic local alignment search tool (BLAST) available at national center software biotechnology information (NCBI) database. The evolutionary relationship of the isolates computed and a phylogenetic reconstructed using Molecular Evolutionary Genetics Analysis (MEGA) software (Version, 6.0). The nucleotide sequence of 16S rRNA gene of each isolated strain were then submitted to NCBI GenBank for obtaining the accession numbers against each sequence.

Preparation of bacterial inoculum

The pure bacterial isolates were grown in nutrient broth for 18–20 h at 37°C and cell pellet was collected after centrifugation at 8000 rpm for five minutes. The cell pellet was washed thrice with sterile normal saline to make it free from media ingredient. These pellets were re-suspended in 5ml of MSM, which served as inoculum for each of the biodegradation experiments.

Biodegradation of Lambda-cyhalothrin

The shake flasks containing MSM and LC (conc. 250 mg/L, w/v) were inoculated with freshly grown bacterial inoculum having growth absorbance of 1.0 at 620_{nm} at a final concentration of 2% (v/v) and bioremediation experiments were run for 20 days at 30°C under agitation (200rpm). The bacterial growth kinetics were recorded every 4th day including zero day of incubation by taking absorbance growth at 620nm spectrophotometer (Jennway 6300, Germany). Control experiments were run without inoculum under similar experimental conditions. All the experiments were run in triplicate and data records were presented as mean of three values \pm standard deviation. The percent removal of LC was determined by using UV-Vis Spectrophotometer (Agilent Technologies, Inc., USA) at 287 nm $(\lambda \text{ max})$. The unknown concentration of residual pesticide (i.e. LC) in the samples was estimated by using equation given below:

$$x = \frac{y + 0.9955}{1.1483}$$

Where, "x" is the unknown concentration in the sample and "y" is the absorbance at 287 nm.

Results and Discussion

Environmental contamination worldwide problem and risk to public wellbeing. These contaminants contain special harmful effects and are responsible for environmental imbalance. These can either be natural pollutants or extraneous substances to contaminate air, water or soil [21]. It is also a matter of apprehension that a few of the contaminants persist for extensive period of time in the environment [22], whereas in additional cases, their derivatives could be more toxic than main contaminants. Acero et al., [23] categorized the pesticides among the major environmental contaminants throughout the world. A few studies illustrated that, a smaller amount as 1% of the total mass of pesticides applied into farming attained its target area. The remaining amount polluted the soil and other ecological like compartments, as air, exterior groundwater as well as causing human health problems due to their persistent nature and toxicity [24]. It is now evident that once the pesticide pollutants persist, they come into contact with water, soil and air; while the native microbes take part in the natural process of biodegradation. Several research workers have reported the degradation of different pesticides under various physiological circumstances and isolated several bacterial strains having potential to degrade the [25]. pesticides from environment researchers have previously reported the isolation of pesticide degrading bacteria from agricultural soil environments [26-27-28-29- and 30]. Similarly the present study was aimed at "bioremediation of LC (pesticide) by bacterial strains Mesorhizobium sp. (S1B) and Bartonella elizabethae (S2B) isolated from cotton crop fields of District this case LC was used as Khairpur. In representatives of pyrethroid pesticides (sole source of carbon and energy) during ex-situ biodegradation studies. It is believed that one gram of soil have approximately more than one hundred million bacteria and 5000-7000 different bacterial strains [31]. The different microbes have been isolated from polluted soils having ability to

degrade pesticides [32-33]. However the isolation pesticide-degrading bacteria by enrichment technique is more preferred over simple isolation techniques and this revealed a positive association between isolation of LCdegrading microbes vs. enrichment technique in MSM [34]. In present study, two different soil samples of cotton crops soil were preferred and selected for bacterial isolation. Moreover, the isolation of LC-degrading bacteria was carried out using enrichment technique on the basis of best growth ODs. Both the soil samples gave significantly higher OD values, as 0.53±0.07 and 0.629±0.02 when compared to control during treatment of 20 days as shown in Fig. 2.

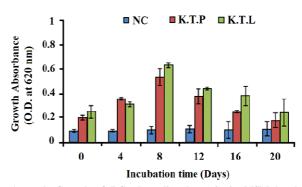


Figure 2. Growth of LC- degrading bacteria in MSM broth supplemented with Lambda-cyhalothrin as sole carbon source. Note: NC= Negative control, K.T.P= Kacha Territory Pir sahib and K.T.L= Kacha Territory Lalan Shah.

The maximum OD was obtained after 8th day of incubation that gradually declined afterwards in both soil samples. The samples showing highest OD_S were selected for the isolation and screening of pesticides degrading bacteria on pesticide containing MSM agar plates. A number of the bacterial strains interfere in the biodegradation course into innate environments cannot be obtained in a laboratory. Though, the isolation of bacteria that may be used in the cleanup of wastes or contaminated environments is very significant [35]. The only two bacterial strains, one from each K.T Pir Sahib and K.T Lalan Shah, were capable of using LC as a source of energy were isolated on MSM agar plates. The pure bacterial isolates were identified by routine microbiological molecular PCR-based characterization methods. The phylogenetic trees and similarity index of the selected bacterial species indicating evolutionary correlation to the closely related bacteria already available in the NCBI GenBank database confirmed isolate S₁B as species of genus *Mesorhizobium sp.* sharing 99% similarity with *Mesorhizobium sp.* STM 4018 gb[EF100516.1] as shown in (Fig. 3). Contrarily, the isolate S₂B shared up to 95% identity with *Bartonella elizabethae* strain R-5 dbj[AB246801.2] and up to 93% with other species of *Mesorhizobium*, thus based on its similarity index, the bacterial isolate S₂B was preliminarily named as *Bartonella sp.*

strain S₂B (Fig. 4). Though, keeping in view of its similarity index, the strain was considered for reclassification and probably renaming as one of the novel species of genus Bartonella (unpublished data). Meanwhile, the nucleotide sequences obtained were submitted to NCBI GenBank database and accession numbers gb|MF471843| gb|MF471844| received were against Mesorhizobium sp. and Bartonella respectively.

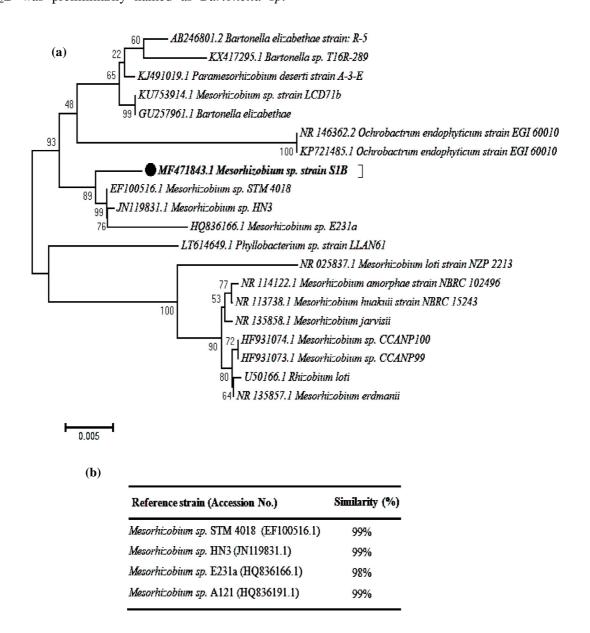
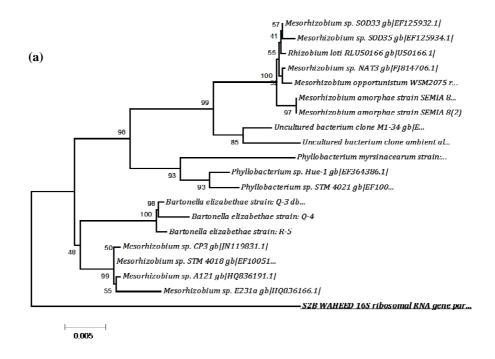


Figure 3. Phylogenic tree (a) and similarity index (b) of Mesorhizobium sp. strain S₁B



(b) Similarity index of *Bartonella elizabethae* strain S₂B.

Reference strain (Accession No.)	Similarity Index
Bartonella elizabethae R-5 (AB246801.2)	95%
Bartonella elizabethae Q-4 (AB246808.1)	94%

 $\textit{Figure 4. Phylogenic tree (a.) and similarity index (b.) of \textit{Bartonella elizabethae} \ strain \ S_2B$

Figure 5 illustrates the growth and LCdegradation by isolate Mesorhizobium sp. (S₁B) and which did not display any prominent log, stationary and decline phases, however the growth OD of strain randomly increased only up to 1.19 \pm 0.06 till last day of incubation. Contrarily, the growth OD of Bartonella sp. (S2B) demonstrated a rapid growth OD 0.93±0.03 till fourth day of incubation that kept on increasing and reached maximum growth OD. 1.13±0.09 during 16 days of incubation. Later, the growth OD of S₂B strain slightly dropped to 1.05±0.07 till the last day of incubation. The biodegradation results of isolate Mesorhizobium sp. (S₁B) displayed 4.98% removal of the LC during 4 days of incubation, and reached 15.11% removal during 8th day of incubation, while removal of LC was found to be 30.78%

during 4th day of incubation with *Bartonella sp.* (S_2B) . The concluding results of growth and biodegradation experiments of LC give explanation that isolate *Mesorhizobium sp.* (S_1B) showed a continuously increasing trend of growth and biodegradation as shown (Fig. 5a), while bacterial isolate *Bartonella sp.* (S_2B) has revealed best growth as well as biodegradation of LC (Fig. 5b).

Evidently, the bacterial isolate *Mesorhizobium sp.* (S₁B) resulted 29.24% percentage removal of pesticide on 20 day of incubation while the isolate *Bartonella sp.* (S₂B) was able to decrease LC concentration 40% in MSM at similar experimental conditions. Selvam, *et al.*, [36] discussed the formation of several

intermediates as well as disappearance of compounds in the degradation of 500ppm of fenvalerate for the treatment of eight days using B. cereus. Jin, et al., [37] reported the degradation rates of a 100 mg/L concentration of βcypermethrin, cypermethrin, fenpropathrin, fenvalerate, and deltamethrin by Acinetobacter sp. strain JN8 in mineral salt medium were 74.1%, 64.9%, 57.9%, 48.1% and 34.9%, respectively. At the optimum growth conditions, Streptomyces parvulus strain (HU-S-01) efficiently degraded 10-250 mg/L cypermethrin within 48 h and completely degraded cypermethrin within 30 h at the concentration lower than 50 mg/L. This is an important feature of an organism to be utilized for biodegradation of cypermethrin residue environment because the reported contamination of cypermethrin in soil or water is not as high as 250 mg/L [38].

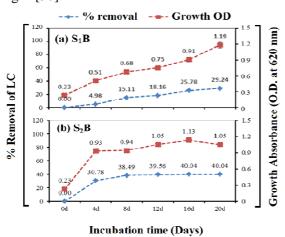


Figure 5. Bioremediation of LC by isolate Mesorhizobium sp.. S₁B (a) and Bartonella alizbathae strain S₂B (b)

Conclusions

The utilization of xenobiotic compounds by soil microorganisms is a crucial phenomenon by which these compounds are removed from the environment, preventing environmental pollution. This research focused on laboratory culture based enriched techniques which have limited the diversity of isolated bacteria. The present study suggests that microorganisms are capable of degrading LC. The bacterial strains *Mesorhizobium sp.* (S₁B) and *Bartonella sp.* (S₂B) provide a sustainable option for improvement of the soil quality because, on one hand, they utilize the

environmental pollutants like LC and, on the other hand making soil more suitable for the crop cultivation by improving its fertility. Additionally, the application of cell free enzymes might be an option for the detoxification of pesticide residues on the surface of fruits and vegetables.

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