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Research in Biotechnology and Environmental Science. 2023; 2(4): 82-87.

DOI: 10.58803/rbes.v2i4.18

http://rbes.rovedar.com/

Research in Biotechnology and Environmental Science

Original Article

Biodegradation of Bifenthrin Using the Bacterium, *Pseudomonas stutzeri* (MTCC2300)

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ARTICLE INFO

Article History:
Received: 04/10/2023
Revised: 05/10/2023
Accepted: 26/11/2023
Published: 25/12/2023

Keywords:
Bacteria
Bioremediation
Degradation
Pesticides
Pyrethroids

ABSTRACT

Introduction: Pyrethroid pesticides are applied in agricultural fields to protect crops from pests and their residues, which can adversely affect soil and water quality, causing damage to non-target organisms. This research aimed to explore the potential role of the bacterial strain, *Pseudomonas stutzeri* in breaking down the pesticide, Bifenthrin.

Materials and Methods: The study focused on evaluating the efficiency of the bacterium, *Pseudomonas stutzeri* (MTCC2300) in degrading the pyrethroid, Bifenthrin. Various concentrations of Bifenthrin (2500, 5000, 7500, and 10000 ppm) were subjected to treatment with the bacterial strain in minimal broth for 16 days.

Results: When the efficiency of *Pseudomonas stutzeri* on the degradation of 2500, 5000, 7500, and 10000 ppm of Bifenthrin was tested for a period of 16 days, a decrease in pH, and an increase in CO_2, NH_3, and biomass were observed. The pH was reduced to 7.6 while CO_2 increased to 4 mg/ml, NH_3 up to 0.8mM, and Biomass up to 0.6 g dry wt./ml. In a two-way ANOVA, Bifenthrin concentration resulted in a statistically significant variation in parameters like, pH, CO_2, and NH_3 of the culture medium.

Conclusion: *Pseudomonas stutzeri* could tolerate Bifenthrin concentrations up to 10000 ppm, and it can be employed in Bioremediation programs for cleaning pyrethroid pesticide-polluted sites.

1. Introduction

Environmental pollution resulting from pesticides, their metabolites, and byproducts poses a threat to biodiversity and natural resources[1]. Pyrethroid pesticides like cypermethrin, deltamethrin, cyphenothrin, and related compounds have been found to have significant adverse effects on the development, behavior, and life of different animals like fish, birds, amphibians, and aquatic mammals[1]. Contamination is directly related to the level of industrialization and intensity of chemical application. Agrochemicals, such as fertilizers and pesticides, are necessary in modern agriculture[2]. While these chemicals provide substantial benefits to crops, their effectiveness is contingent on remaining in the root zone of the soil. If they reach non-target organisms, they cause harmful effects on the environment[3]. They may leave the root zone through volatilization to the atmosphere, leaching to the subsoil and groundwater, runoff to surface water systems, and plant uptake[4,5]. Pesticides exert an influence on soil microbes and alter the chemical characteristics of the soil, persisting in the soil environment. They are toxic and their residues exhibit bioaccumulation and affect beneficial insects and birds[6,7]. Further, continuous use of pesticides creates resistance to their action on pests and leads to further modification, while production of new pesticides causes pollution during production, use, and after application[8,9].

Bifenthrin is one of the most familiar pyrethroids available for successfully controlling pests of several

▸ *Cite this paper as:* Sabitha, Surendran A, Thatheyus AJ. Biodegradation of Bifenthrin Using the Bacterium, *Pseudomonas stutzeri* (MTCC2300). Research in Biotechnology and Environmental Science. 2023; 2(4): 82-87. DOI: 10.58803/rbes.v2i4.18

commercially important crops[10]. It is also widely applied in urban areas to control household pests, such as termites[11]. Based on the nature of soil, moisture, pH, and environmental conditions, the half-life period of Bifenthrin varies from a few weeks to more than a year. It is grouped as a toxicity class II moderately hazardous pesticide[12]. Among various pyrethroids, Bifenthrin leaves more toxic residues found in urban areas[13]. Hence, Bifenthrin severely impacts water and soil contamination, which necessitates finding a suitable method to reduce its toxic effect on the environment.

Microbial degradation is commonly employed for pesticide removal due to its cost-effectiveness and low hazards to indigenous animals and plants[14]. Degradation of pesticides usually accompanies a number of processes, like chemical hydrolysis and microbial degradation, and is also influenced by certain physico-chemical characteristics like pH, organic carbon, and moisture content. However, microbial degradation is the main mechanism of pesticide breakdown and detoxification in soil. Hence, microbes affect the persistence of most pesticides in soil[15]. *Pseudomonas stutzeri* (*P. stutzeri*) has emerged as a promising bacterium for the biodegradation of Bifenthrin, a widely used pyrethroid insecticide[16]. The metabolic versatility, biodegradative efficiency, environmental adaptability, safety profile, and genetic manipulability of *P. stutzeri* make it a valuable tool for bioremediation of bifenthrin-contaminated sites. Its potential to effectively remove this persistent insecticide from the environment underscores its significance in environmental protection and sustainable agricultural practices[16]. The selected bacterial strain can tolerate even 10000 ppm of Bifenthrin. In this context, the present study aimed to evaluate Bifenthrin degradation efficiency using *P. stutzeri* (MTCC2300) and also to assess the changes in pH, releasing CO_2, producing NH_3 and biomass during degradation.

2. Materials and Methods

2.1. Pesticide

The synthetic pyrethroid, Bifenthrin (2 methyl biphenyl–3–ylmethyl-[Z]-[1RS]–3-[2-chloro-3,3,3 trifluoro prop1enyl]-2, 2- dimethyl cyclopropane carboxylate) from Aristo Biotech and Life Science (P) Limited (Vadodara, Gujarat, India) was chosen for this study based on its wide extent of application within agricultural fields and present market trends.

2.2. Degradation efficiency

The bacterium, *P. stutzeri* strain (Microbial Type Culture Collection [MTCC] 2300) was obtained from the Institute of Microbial Technology (IMTECH), Chandigarh, Punjab, India. It was inoculated as 1 ml (10^9 cells) from the logarithmic phase of the pure culture grown in nutrient broth into minimal broth having different concentrations of 2500, 5000, 7500, 10000 ppm of commercial-grade raw pesticide Bifenthrin. The flasks were then incubated at 37°C for 20 days. The samples taken were then subjected to the estimation of pH, CO_2 released, NH_3 produced, and biomass[7]. All the values calculated represent the means of three observations.

2.3. Estimation of pH

The pH in the chosen test concentrations was determined by employing a pH meter every day for 20 days.

2.4. Estimation of carbon dioxide

Free CO_2 was determined by titrating the four samples employing a strong alkali (NaOH) to pH 8. Sodium hydroxide was prepared in CO_2-free distilled water (boiled), from which 50 ml was diluted in 1000 ml CO_2-free distilled water and titrated against 100 ml of the sample. Phenolphthalein was utilized as the indicator, and the pink color signaled that the endpoint had been reached. The free CO_2 was decided utilizing the following formula.

$$\text{Free } CO_2 \text{ (mg/ml)} = \frac{\text{Titre value x Normality of NaOH x 1000 x 44}}{\text{Vol. of sample}}$$

2.5. Estimation of ammonia

In this stage, 1 ml of sample was taken in a test tube, and a number of drops of Nessler's reagent were added, and the OD was examined at 660 nm.

2.6. Estimation of biomass

The biodegraded samples were taken and centrifuged. The pellet was collected and poured into a Petri dish. Then, the Petri dish containing the pellet was dried in a hot air oven at 80°C for 3 hours. The final dried biomass was weighed, and dry biomass was determined.

2.7. Statistical analysis

Two-way ANOVA was performed on the parameters, such as ammonia, biomass, pH, and carbon dioxide, utilizing Microsoft Excel (Version: 12.0.6611.1000). Variability was measured as statistically significant only when the calculated F value was higher than the tabulated F value at p is less than (or) equal to 0.05.

3. Results

The biodegradation of Bifenthrin by *P. stutzeri* was examined, and the changes in CO_2 released, pH, ammonia production, and biomass during the degradation were studied. Figure 1(a) indicates the effective degradation of Bifenthrin by *P. stutzeri* at a pH of 7-8. With the enhancement in the treatment period, the pH was shown to reduce. The biodegradation of Bifenthrin led to an increase in CO_2 released in the medium, which was directly proportional to the Bifenthrin concentration. During the degradation of 10000 ppm of Bifenthrin by *P.stutzeri,* more CO_2 was released. The amount of CO_2 released after 16 days of treatment of Bifenthrin by *P. stutzeri* is shown in Figure 1(b). In that, the maximum amount of CO2 released for Bifenthrin was 10000 ppm. The release of ammonia as a result of biodegradation of Bifenthrin by *P. stutzeri is* shown in Figure 1(c). The concentration of liberated ammonia increased on days 4 and 8 and decreased on days 12 and 16 of treatment for various Bifenthrin concentrations by the bacterial strain. The maximum ammonia release was observed during day 4 of Bifenthrin treatment by *P. stutzeri.* Figure 1(d) delineates the changes in biomass of *P. stutzeri* during the study period. There was a gradual enhancement within the biomass of the bacterial strain, showing an enhancement in bacterial growth utilizing Bifenthrin as a source of carbon and energy.

The two-way analysis of variance for the factor pH with the variables, treatment period, and Bifenthrin concentration for *P. stutzeri* is shown in Table 1. The variation due to Bifenthrin concentration was statistically significant at the level of 5%, whereas for the treatment period, it was not statistically significant. The two-way analysis of variance for the factor carbon dioxide released with the variables, treatment period, and Bifenthrin concentration for *P. stutzeri* indicated that the variations due to Bifenthrin concentration were statistically significant ($p < 0.05$), and variation due to treatment period was not significant ($p < 0.05$). The two-way analysis of variance for the factor ammonia with the variables, treatment period, and Bifenthrin concentration is exhibited in Table 1. The variation due to the treatment period was not statistically significant, and the Bifenthrin concentration was statistically significant at 5%. The two-way analysis of variance for the factor biomass with the variables such as Bifenthrin concentration and treatment period has shown that the variations due to Bifenthrin concentration and treatment period were statistically significant ($p < 0.05$).

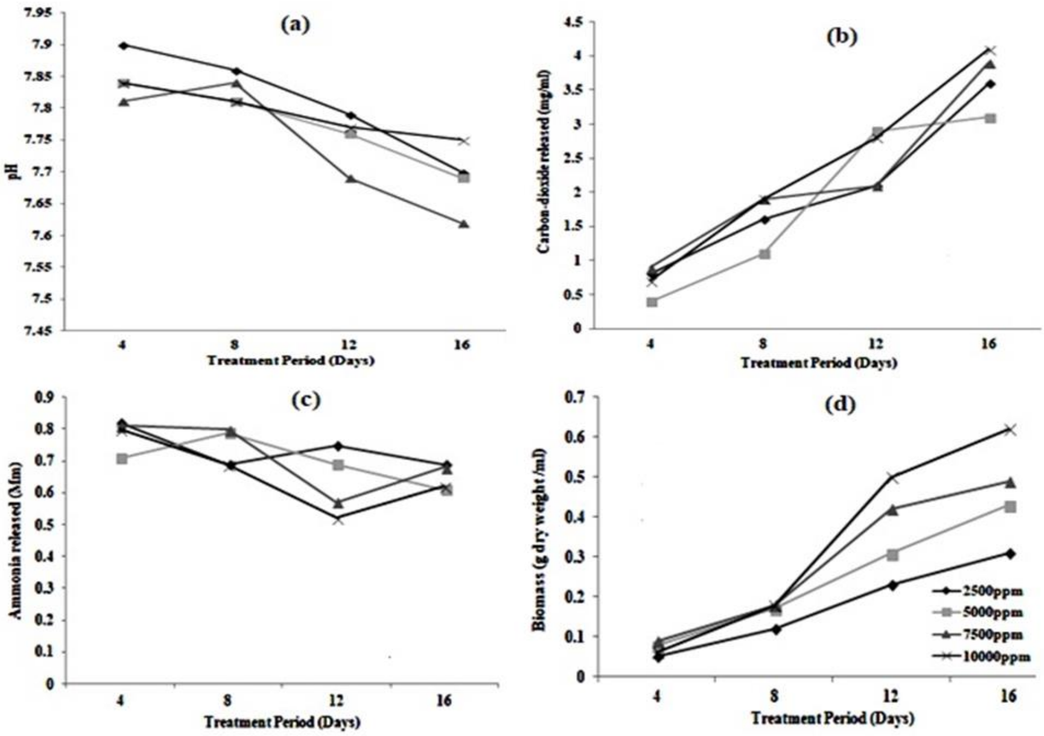

Figure 1. Changes in pH (a) Carbon-dioxide released (b) Ammonia released (c) and Biomass (d) during the biodegradation of Bifenthrin using *Pseudomonas stuzeri* (MTCC 2300)

Table 1. The variations amid the biodegradation of Bifenthrin utilizing *Pseudomonas stutzeri* (MTCC2300)

Item	Source of Variation	Sum of Squares	Mean Sum of Squares	Calculated F -Value	Table F -Value	P value
pH	Concentration	0.064	0.021	21.217	3.863	Significant [p<0.05]
	Treatment Period	0.011	0.004	3.783	3.862	Not Significant [p>0.05]
CO_2	Concentration	19.229	6.407	50.556	3.863	Significant [p<0.05]
	Treatment Period	0.562	0.187	1.478	3.863	Not Significant [p>0.05]
Ammonia	Concentration	0.064	0.021	4.401	3.863	Significant [p<0.05]
	Treatment Period	0.014	0.005	0.935	3.863	Not Significant [p>0.05}
Biomass	Concentration	0.390	0.130	30.883	3.863	Significant [p<0.05]
	Treatment Period	0.058	0.019	4.587	3.863	Significant [p<0.05]

4. Discussion

The use of pesticides in agriculture contributes to increased agricultural productivity. However, only a small percentage of pesticides is required to kill the pests. The remaining pesticides create various kinds of pollution[17]. In agriculture, the utilization of pesticides should include a pest control and biological control strategy, culture strategies, pest monitoring, and other methods known as integrated pest management. Pesticide selection should be based on efficacy, non-toxicity to other species, low cost and field characteristics, solubility, and persistence[18]. Since pests are one of the main causes of economic loss in agriculture, the utilization of pesticides is essential[18,19].

Pesticides are widely used for pest control in agriculture. Therefore, the illegal use of pesticides has caused serious problems not only to humans but also to other organisms living in the environment[20,21]. Environmental pollution caused by pesticides affects nearby ecosystems. Pesticides sprayed on the surface of agricultural fields can travel long distances, seep, and contaminate the water table in visible concentrations. Therefore, the decontamination of areas contaminated by pesticides is a difficult task[22].

Microorganisms provide the opportunity for biodegradation of pesticides. The ability to reduce xenobiotic concentrations is consistent with long-term adaptation to a polluted environment. In addition, genetic engineering can improve the performance of microorganisms that possess the positive properties necessary for biodegradation[23]. Recent studies indicated that microbial degradation to remove pesticide residues can avoid contamination problems[24]. Bacterial strains that can degrade many pesticides include a chlorpyrifos degrading *Pseudomonas sp.*[25], a metamitron degrading *Rhodococcus sp.*[26], and an iprodione degrading *Arthrobacter sp*[27]. It has been indicated that bacteria with the ability to degrade some pesticides can be used for the bioremediation of pesticide-contaminated sites. In the present study, *P. stutzeri* was examined for its efficiency in degrading Bifenthrin.

Bifenthrin can bind to soil particles and cannot move through the soil. Since it is poorly soluble in water, it will stick to the soil and cause little pollution to groundwater. However, it binds to soil and contaminates surface water through runoff[28]. The half-life in the soil varies from 97 to 250 days, depending on the soil type. The half-life of Bifenthrin in the soil is 106 to 147 days when the soil is exposed to sunlight. Bifenthrin has a photodegradation half-life of more than 100 days and is therefore considered stable[29].

Bifenthrin is photostable with aqueous photolysis and has greater insecticidal activity than other pyrethroids. Due to the low vapor pressure, it is unlikely to evaporate from dry soil. However, it may be slightly more common in wet soils than in dry soils. It is a member of the class of synthetic pyrethroid family of pesticides[30]. Like most pyrethroid pesticides, Bifenthrin affects the central and peripheral nervous system of insects, causing paralysis. Bifenthrin is so toxic to aquatic life that it is listed as a "restricted use pesticide" for sale and use only by authorized applicants[10].

Pseudomonas stutzeri indicates favorable degradation efficiency compared to other biodegradation methods, presenting distinct advantages over conventional physico-chemical methods. Fungi can also eliminate Bifenthrin, but their degradation rates are generally slower than that of *P. stutzeri*. In addition, fungi are often more sensitive to environmental factors, such as temperature and pH. Algae have been found to degrade Bifenthrin, but its effectiveness is limited because it requires sunlight. This makes them unsuitable for bioremediation in shaded or subsurface environments. Although many other bacteria have been shown as potential bifenthrin degraders, *P. stutzeri* has consistently demonstrated superior degradation efficiency and adaptability[23,26]. The increase in biomass during the treatment period indicates the growth of bacteria resulting from the decomposition of pesticides as a source of carbon and nitrogen. In general, an increase in pesticide exposure correlates positively with an increase in incubation time[31]. The release of carbon dioxide and ammonia during pesticide decomposition indicates bacterial activity in the environment[32]. In the present study, the maximum release of carbon dioxide was observed at 10000 ppm and ammonia at 5000 ppm during Bifenthrin degradation by *P. stutzeri*.

Many microorganisms have been reported to use pesticides as a source of energy. The fungi include *Trametes hirsutus, Phanerochaete chrysosporium, Phanerochaete sordia,* and *Cyathus bulleri,* which can degrade pesticides[33]. However, most evidence indicates that soil bacteria play an important role in biodegradation. When microorganisms encounter a new organic molecule, they may acquire new catabolic genes from other microbes through conjugation or transformation or modify existing genes through mutation[34]. During this process, the bacterium undergoes an adaptation. Microbes that have been exposed to synthetic chemicals have developed the ability to use some of them. Several types of bacteria have been indicated to degrade xenobiotics. Most of these xenobiotic-degrading bacteria harbor plasmids encoding catabolic genes[35]. By understanding the biochemistry and genetics of plasmid-mediated degradation and using recombinant DNA technology, appropriate genes can be identified and transferred to create better and more efficient strains to reduce some toxic pesticides[36].

Pseudomonas stutzeri is a natural microorganism, and its use for bioremediation presents very low environmental risks compared to physicochemical methods that contain hazardous substances or produce by-products. Bioremediation using *P. stutzeri* is generally more cost-effective than physicochemical methods, particularly for large-scale remediation projects. Bioremediation with *P. stutzeri* promotes sustainable environmental management using natural methods to restore contaminated sites[34].

Biodegradation of Bifenthrin by *P. stutzeri* decreases the persistence of this insecticide in the environment, reducing the risk of contaminating non-target organisms and altering ecosystems. Bioremediation using *P. stutzeri* can improve soil quality by removing Bifenthrin and other pollutants, making it more suitable for plant growth and ecosystem health. However, its ability must be verified in field conditions. Biodegradation of Bifenthrin in contaminated water bodies helps to protect aquatic ecosystems and prevents the spread of the insecticide to downstream areas. Bioremediation with *P. stutzeri* offers a sustainable alternative to conventional pesticide use, reducing reliance on chemical applications and promoting environmentally friendly agricultural practices[35].

5. Conclusion

The findings suggest that *P. stutzeri* can be used in bioremediation programs to degrade pyrethroid pesticides and their residues. In minimal broth, *P. stutzeri (MTCC2300)* could degrade Bifenthrin up to 10000 ppm concentration. The reduction in pH confirmed the degradation and increase in the amount of CO_2, NH_3, and biomass after sixteen days of treatment.

Declarations

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

Sabitha designed the study methodology, performed the statistical data analysis, and conducted the literature reviews. Appasamy Surendran prepared and critically revised the research manuscript. Antony Joseph Thatheyus guided on data analysis and interpretation of results findings, and literature review. All the authors read, substantially revised, and approved the final edition of the manuscript.

Funding

The authors received no financial support for the present study.

Availability of data and materials

The manuscript contains all datasets generated and/or analyzed in the current study.

Ethical considerations

The authors checked for plagiarism and consented to the publishing of the article. The authors have also checked the article for data fabrication, double publication, and redundancy.

Acknowledgments

The authors thank the authorities of The American College, Madurai, Tamil Nadu, India, for the facilities and encouragement.

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