VOLUME 8 ISSUE 2 2022

ISSN 2454-3055

INTERNATIONAL JOURNAL OF ZOOLOGICAL INVESTIGATIONS

Forum for Biological and Environmental Sciences

Published by Saran Publications, India

International Journal of Zoological Investigations Vol. 8, No. 2, 211-217 (2022)

International Journal of Zoological Investigations
Contents available at Journals Home Page: www.ijzi.net
Editor-in-Chief: Prof. Ajai Kumar Srivastav
Published by: Saran Publications, Gorakhpur, India

ISSN: 2454-3055

Biodegradation of Synthetic Pyrethroid Insecticide Deltamethrin by *Stenotrophomonas maltophilia* Strain DRNB1

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Received: 28th June, 2022; **Accepted:** 2nd August, 2022; **Published online:** 8th August, 2022

https://doi.org/10.33745/ijzi.2022.v08i02.027

Abstract: Deltamethrin is one of the most used pyrethroid insecticide in different regions of the world. It is the fast-acting insecticide that disrupts the cellular sodium channels. The deltamethrin residues are highly retained in environments, particularly in soil and water and have inevitable side effects on natural resources and human health. The most efficient, economical, and environmentally beneficial way of removing deltamethrin from contaminated sites is bioremediation. Bacteria are the most used biological agents in biodegradation studies. Thus, in this study deltamethrin-degrading bacteria *Stenotrophomonas maltophilia* strain DRNB1 was isolated and characterised. The bacterial growth was analysed by UV-spectrophotometer and deltamethrin degradation was studied by GC-MS analysis. *S. maltophilia* strain DRNB1 used deltamethrin as the sole carbon source for growth. Deltamethrin degradation efficiency of DRNB1 was 89.2% in insecticide supplemented media and 93% in presence of additional glucose in mineral media. These results implies that *S. maltophilia* strain DRNB1 could be used as a bioremediation technology for deltamethrin contaminated environments.

Keywords: Deltamethrin, *Stenotrophomonas maltophilia* DRNB1, Bioremediation, Soil, Pyrethroid

Citation: Josna Victoria K. Johnson and John Vimala K.: Biodegradation of synthetic pyrethroid insecticide deltamethrin by *Stenotrophomonas maltophilia* strain DRNB1. Intern. J. Zool. Invest. 8(2): 211-217, 2022.
https://doi.org/10.33745/ijzi.2022.v08i02.027

Introduction

Pesticides are essential components of many agricultural management systems, and they can be used to control rodents, fungal infections, and weeds. Insecticides are used to reduce the spread of insect-borne diseases and agricultural pests in public health and agriculture, respectively (Damalas and Eleftherohorinos, 2011). The continued and widespread use of agrochemicals has negative impact on human health and causes several environmental consequences (Piutti *et al*., 2002).

Deltamethrin - (s) – alpha – cyano – 3-phenoxy benzyl (1R,3R)-3-(2,2-dibromovinyl)-2,2-dimethyl cyclo propanecarboxylate) is a commonly used pyrethroid insecticide around the world with a broad spectrum of applications, primarily as an

acaricide and scabicide. They are synthetic substances designed to look like the pyrethrins found in chrysanthemum blooms. Because of its low cost, persistence, and durability, it has been widely used for more than 30 years all over the world. It is used to control pests such as mites, ants, weevils, and beetles on a variety of crops including cotton, corn, cereals, and vegetables since it is effective at low concentrations. Deltamethrin, which is particularly lipophilic, easily penetrates the cuticles of insects and acarines and kills them through ingestion. It functions as a neutrotoxin, causing the sodium channel activation gate to be inhibited. This interacts with y-aminobutyric acid receptors, causing neuronal transmission to be blocked (Ecobichon, 1991). Because of their phosphorylation, it also affects the action of chloride and calcium channels (Burr and Ray, 2004). It is identified in various environmental matrices as a result of persistent application, particularly in soil and water, where it can be hazardous to both target and non-target organisms (Hintzen *et al*., 2009).

The increased use of synthetic pyrethroids like deltamethrin have adverse health effects to human such as lymph node and splenic damage, carcinogens, and hormonal activity. Pyrethroids have cumulative neuroreproductive toxicity and endocrine disruption effects on nontarget animals (Hintzen *et al*., 2009; Kawahara *et al*., 2010). Chronic diseases may result from long-term exposure to certain insecticides (Osman and Abdulrahman, 2003). Some of them are considered to be carcinogenic (Pankaj *et al*., 2013). All of these elements combine to make pyrethroids potentially hazardous to human health and the environment. As a result, remediation measures to breakdown and eradicate pyrethroid residues from the environment are required. Bioremediation is considered as an environmentally and economically sustainable technology used for the removal of hazardous contaminants.

Soil microorganisms play a key part in this biodegradation process of deltamethrin (Chapman *et al*.,1981; Zhang *et al*.,1984; Grant and Betts, 2004). The aim of the present study was to isolate and characterize the deltamethrin degrading bacteria from tea garden soil.

Materials and Methods

Chemicals and media:

The pesticide used in the current study was reference standard deltamethrin with purity of (98%) obtained from Himedia. The chemical was dissolved in acetone for further studies. In the present study, degradation of the target compound (deltamethrin) only was studied, not its metabolites. All other chemicals were of analytical grade.

The MSM media for enrichment culture containing $2SO_4$ 2.0 g/l, KH_2PO_4 1.5 g/l, $CaCl_2.H_2O$ 0.01 g/l, $Na_2HPO_4.12H_2O$ 1.5 g/l was autoclaved (121 ^oC, 15min) and cooled. The media was supplemented with 50 mg/l of deltamethrin as the sole source of carbon and nitrogen is used. The pH of the medium was adjusted to 7.2 to evaluate biodegradation (Chen *et al*., 2011a, 2012).

Isolation of deltamethrin degrading bacteria:

The enrichment culture techniques were used for the isolation of deltamethrin degrading bacteria. For this purpose 20 g of tea garden soil of Annamalai hills region of Western Ghats (previously treated with deltamethrin) was added to flasks containing 100 ml of MSM supplemented with 50 mg/l of deltamethrin. Samples were incubated for 72 h on a rotary shaker (120 rpm) in a darkened thermostatic chamber maintained at 30±2°C after incubation, about 1 ml of soil suspension was transferred into flasks containing fresh MSM with the same concentration of deltamethrin and then incubated for an additional 72 h under the same condition. After seven subsequent transfers into the same medium, serial dilutions of the flask samples were plated onto MSM agar plates supplemented with 50 mg/l of deltamethrin to isolate the individual colonies (Cycon *et al*., 2014). Isolates exhibiting distinct

colony morphologies were isolated by repeated streaking on the same MSM agar medium.

Identification of bacterial isolates:

Isolates were characterised and identified using morphological features, biochemical tests and 16S rRNA gene analysis. The biochemical tests done were oxidase test, catalase tests, Lactose fermentation, motility test, gram staining, sulfide indole test and methyl red test.

Biodegradation experiment:

(A) *Inoculum preparation*:

Deltamethrin degrading strain designated as DRNB1 was used for the inoculum preparation. The bacterial strain was cultured in 100 ml Erlenmeyer flasks containing 20 ml of MSM supplemented with 50 mg/l of deltamethrin. The bacteria were pelleted by centrifugation (5 min, 10000 g) at the exponential phase. The pellet was washed twice with 0.85% of sterile NaCl and then resuspended in NaCl to obtain the bacterial suspension at a concentration of approximately 3 $x10^8$ cells/ml. The cell density (OD at 660 nm) was measured using a UV-VIS spectrophotometer.

(B) *Biodegradation of deltamethrin in MSM*:

The degradation studies were performed in 500 ml Erlenmeyer flasks containing 200 ml of sterile MSM supplemented with deltamethrin as the only source of carbon. The amount of insecticide applied is 50 mg/l. The medium was inoculated with 1 ml of bacterial suspension giving a final concentration of approximately $3x10^9$ cells/ml. Triplicate samples of MSM with strain DRNB1 as well as insecticide only used as controls. All samples were incubated on a rotary shaker (120 rpm) maintained at 30±1°C. Samples were removed periodically for bacterial growth rate and to determine deltamethrin concentrations. The growth of bacterial strains was recorded spectrophotometrically by measuring the OD at 660 nm using a UV-VIS spectrophotometer (Cycon *et al*., 2014).

(C) *Chemical analyses*:

To determine the deltamethrin concentration 10 ml of MSM was taken for analysis. Samples of MSM were filled to volume of 20 ml with deionised water and extracted twice with ethyl acetate. The extracts were dehydrated with anhydrous Na_2SO_4, evaporated to dryness under a stream of N_2 at 45°C using rotary evaporator and diluted to a final volume of 10 ml with hexane, and reserved for chromatographic analysis. Concentrations of deltamethrin were determined by gas chromatography. GC-MS column (Rxi 5SilMS), carrier gar helium and software GCMS solutions.

Results and Discussion

Isolation and characterization of deltamethrin degrading bacteria:

Four morphologically different bacterial isolates were obtained from tea garden soil through enrichment culture. The four isolates were selected to screen the deltamethrin degradation potential. The bacterial strain DRNB1, showed high tolerance to insecticide deltamethrin (50 mg/l) as a result, the strain DRNB1 was selected for further studies. The tea garden soil strain DRNB1 used deltamethrin as sole carbon source. The morphological, physiological and biochemical characterisation of bacterial strain DRNB1 was done. The characteristics were presented as follows: Gram negative rod shaped, non-motile, aerobic bacteria ,nitrate reduction test positive catalase positive, oxidase negative and mobile organism (Table 1).

Table 1: Morphological and Physiological characters of *S. maltophilia* strain DRNB1

Biochemical tests	Results
Shape	Rod shaped
Colony color	Bright yellow changes to dark brown
Gram staining	Negative
Motility	Non-motile
Catalase	+ve
Oxidase	+ve (slightly)
Nitrate reduction test	+ve

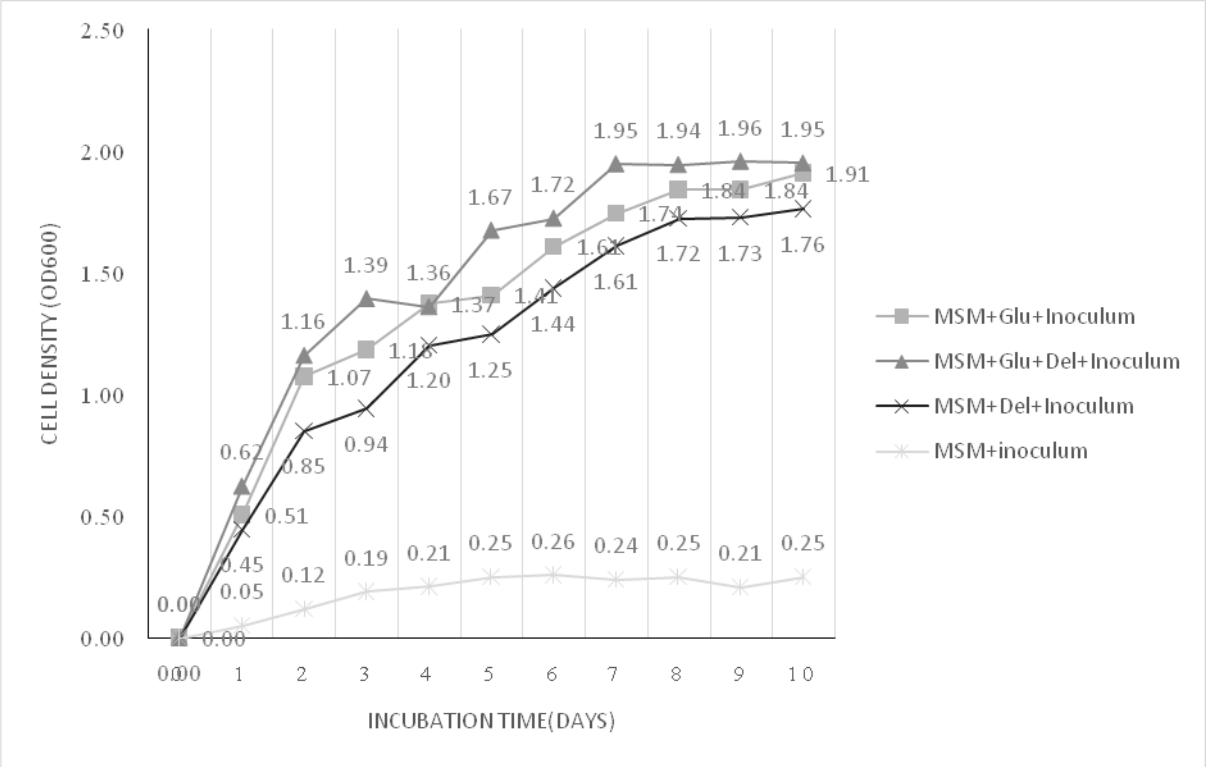

Fig. 1: Growth of bacterial strain DRNB1 in different Mineral Salt Media. MSM-Mineral Salt Media, Glu-Glucose, Del- Deltamethrin and Inoculum- DRNB1.

The 16S rRNA partial gene amplification of strain DRNB1 (733 bp) and BLAST search showed the similarity between DRNB1 and *Stenotrophomonas* sp. (accession number – CP052863.1). Therefore, this tea garden soil strain was named as *Stenotrophomonas maltophilia* DRNB1 and deposited in GenBank under the accession number ON384040.

Degradation of deltamethrin by S. maltophilia DRNB1 in MSM:

The growth of bacteria is also increased in the presence of glucose. Maximum bacterial growth, cell density 1.94 was obtained on the 8th day (Fig. 1). The degradation rate of deltamethrin in the control flasks (MSM and deltamethrin, without the strain DRNB1) was about 15% after 10 days of incubation (Fig. 2).

The addition of glucose increased the growth of bacteria and biodegradation of deltamethrin. In the absence of glucose, the deltamethrin degradation efficiency of *S. maltophilia* DRNB1 was 89% at the end of 10 days. The addition of 1 g/l of glucose increased the deltamethrin degradation efficiency of *S. maltophilia* to 92%, which corresponds to an increase of 2.8% (Fig. 2).

The *Stenotrophomonas* species has been shown to degrade the variety of hazardous compounds like polycyclic aromatic hydrocarbons (Juhasz *et al*., 2000), acrylamide (Lakshmikandan *et al*., 2014), acetamiprid (Tang *et al*., 2012), endosulfan (Barragain-Huerta *et al*., 2007; Kumar *et al*., 2007) a wide range of pyrethroids such as fenvalerate, deltamethrin, β-cypermethrin and cyhalothrin (Chen *et al*., 2011b), 4-substituted phenols (Liu *et al*., 2009), herbicide butachlor (Dwivedi *et al*., 2010), diuron (Batisson *et al*., 2007; Egea *et al*., 2017; Silambarasan *et al*., 2020), α-endosulfan (Ozdal *et al*., 2017) and diazinon (Pourbabaee *et al*., 2018). Gur *et al*. (2014) reported that *S. maltophilia* OG2 could degrade 69.9% of 100 mg/l cypermethrin after 10 days incubation. Wu *et al*. (2021) reported that *S. maltophilia* XQ08 could degrade 63.26% of 100 mg/l deltamethrin after 5 days incubation. An inoculation of soil contaminated with fenvalerate (50 mg/kg of soil) with *Stenotrophomonas* sp.

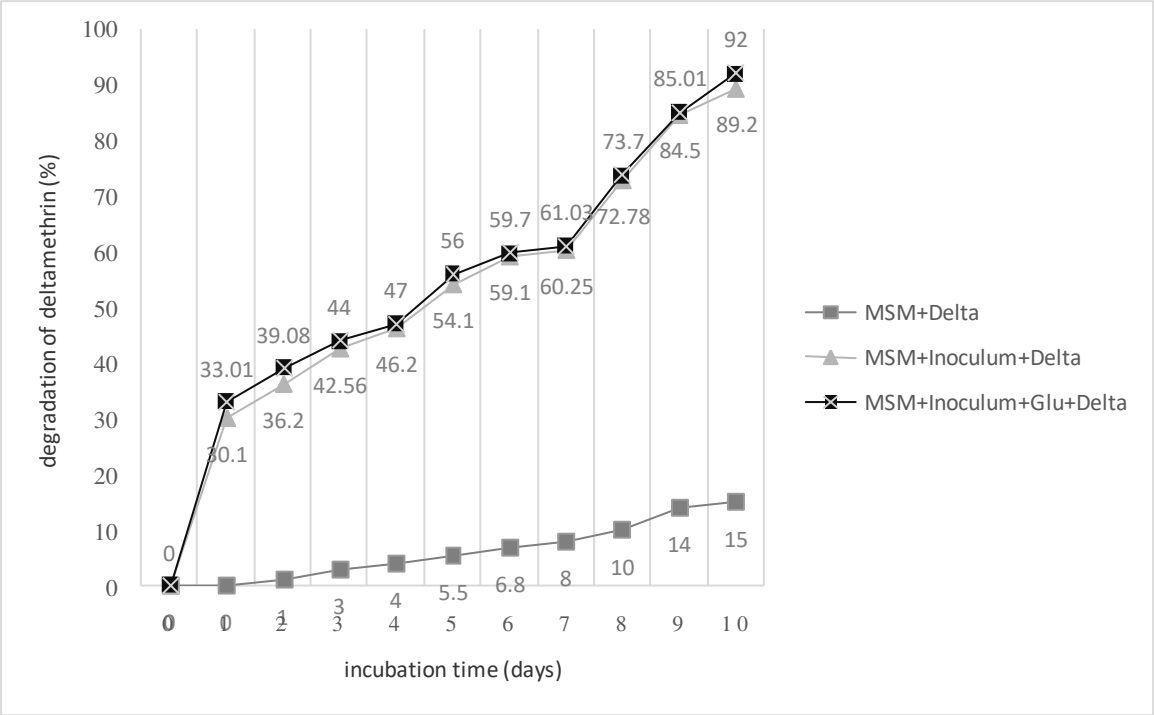

Fig. 2: Degradation of deltamethrin in Mineral Salt Media supplemented with glucose and deltamethrin. MSM with deltamethrin without DRNB1 is taken as control.

Strain ZS-S-01 increased the rate of pyrethroid dissipation, and its half life value was eight times lower than for soil without strain (Chen *et al*., 2011b).

Bacteria capable of degrading deltamethrin were mostly isolated from agricultural areas where intensive pesticides were used. Chen *et al*. (2012) found that *Streptomyces aurus* HPS-01 could degrade cypermethrin. Song *et al*. (2015) studied the deltamethrin biodegradation with *Pseudomonas aeruginosa* JO-41 strain isolated from the pyrethroid contaminated soil. *Acinetobacter calcoaceticus* MCm5, *Brevibacillus parabrevis* FCm9, *Sphingomonas* sp. Rcm6, *Bacillus megaterium* JCm2, *Ochrobactrum anthropic* JCm1 and *Rhodococcus* sp. JCm5 were used in biodegradation of deltamethrin (Akbar *et al*., 2015a, b). Cycon *et al*. (2014) isolated deltamethrin degrading *Serratia marcescens* Del-1, Del-2 from insecticide treated soil. The other deltamethrin degrading species are *Bacillus cereus* Y1 (Zhang *et al*., 2016), *Lysinibacillus fusiforms* ZJ6 (Hao *et al*., 2018), *Acinetobacter baumannii* ZH-14 (Zhan *et al*., 2018). Kumral *et al*. (2020) reported deltamethrin degrading *Lactobacillus plantarum*, which is used as a food fermenter. The efficiency of pesticide degradation increased with the addition of extra carbon sources (Kumar and Philip, 2006; Chen *et al*., 2011a, 2012).

Conclusion

In this study, a deltamethrin degrading bacterial isolate *Stenotrophomonas maltophilia* DRNB1, was isolated from tea garden soil by enrichment technique. Strain DRNB1 utilizes deltamethrin as a sole source of carbon. Environmental problems due to pesticide contamination is one of the major problems. Biodegradation of the pesticides is the efficient method to remediate pollutants from the contaminated sites. Therefore, this study showed that *Stenotrophomonas maltophilia* DRNB1, can be used as a bioremediation tool to eliminate contaminants from polluted sites.

Acknowledgements

This research was supported by UPASI Tea Research Foundation, Valparai helping in soil sample collection. Thanks are extended to Head, The Research and Post Graduate Department of

Zoology, St.Thomas' College, Thrissur, Kerala for providing lab facilities.

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