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Optimization of Biodegradation of Cypermethrin by *Bacillus stercoris* isolated from pesticide-polluted farm soil and analysis of the metabolic products using GC MS

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ABSTRACT

Pesticides are frequently used to control pests in residential gardens, veterinary clinics, and agricultural settings. Because of their widespread use, they increasingly pose greater dangers to human-associated non-targeted organisms. Cypermethrin is popularly used for controlling different pests in various stages of the development of crops. Excess use of it causes soil and water pollution, and this affects soil microflora and aquatic life, too. There are different methods available for cypermethrin removal from the environment, such as Physical and chemical methods, but most of them are time-consuming and very expensive.

The goal of the current study is to isolate microorganisms from polluted soil that are resistant to high cypermethrin concentrations. To isolate the microorganisms, the enrichment culture method was employed. Bacteria that grow on minimum media with cypermethrin were eliminated and identified using their morphological and biochemical characteristics. According to phylogenetic analyses, one bacterial isolate (PRSB-1) belonging to *Bacillus stercoris,* along with four other bacterial isolates (PRS-1, PRS-2, PRS-3, PRSB-2) are from *Pseudomonas, Klebsiella, Bacillus,* and *Lactobacillus spp.* respectively. The optimum conditions required for the growth and degradation of pesticide were studied, and it was found that 40°C temperature and 7 pH is most suitable for degradation. Isolated members of the genera *Bacillus* were utilised for more thorough deterioration investigations with GC-MS analysis. The results of the degradation study unequivocally show that in situ bioremediation is the optimum method for this organism.

INTRODUCTION

It is evident that the need for food grains has increased, and food loss as a result of different pests has been noted and experienced. Pesticides are frequently used to manage household and agricultural pests to prevent such losses. Pesticides have significantly decreased food loss, yet they are still widely present in soil, water, air, and agricultural goods. Therefore, there is a significant risk to the ecosystem from the widespread use of pesticides (Chen, Sun, 2006; Fenner, Canonica, 2013). They directly endanger human health and the ecosystem by polluting not just the soil and crops but also groundwater and the marine environment (Mrema, Rubino, 2013; Nayak, Dash, 2018).

There are several types of pesticides used in the agriculture sector. Each type has its modes of action to kill pests, and their metabolism in soil may be different. Synthetic pyrethroids are different from other types of pesticides because they contain chiral centers in their structure. Researchers have proven that biodegradation of pyrethroids is possible to convert it to the safe and environmentally friendly form (Laskowski, 2002). Also, pyrethroids are safer than other types, but the uncontrolled applications of these pesticides are leading to extensive contamination of the environment. Studies have shown that the pyrethroids have a negative impact on the environment, and aquatic animals, insects, and other animals are also affected. In the case of human beings, the endocrine system, immune system, reproductive system, and overall health of people are also affected (Zhang et al., 2010).

It is very important to develop quick and effective methods to remove pesticides and insecticides from the environment. There are many methods available, but the most promising and effective method is the use of bacteria, and biodegradation is the most suitable approach (Chen et al., 2012). A variety of microorganisms, such as bacteria, fungi, actinomycetes, and even algae from natural sources, have the ability to metabolize the pesticides. Amongst them, bacteria can adapt and can be mutated for degradation of pesticides. Several bacteria, such as *E. coli*, species of *Bacillus*, *Thiobacillus*, and many more, can degrade pesticides (Kafilzadeh, 2018).

Microorganisms can be isolated from natural sources. The provision of optimum conditions leads to the suitability for growth and degradation environment. Hence, a variety of conditions are provided for growth and degradation to the isolated bacteria, and an optimization study was carried out in the present study. The bacteria were isolated, characterized morphologically, identified by the 16s rRNA sequencing method, and then given exposure to different conditions for the optimization study and degradation study. Further, the metabolic products were identified by GC MS analysis.

2. Materials and Methods:

Chemicals and media

The synthetic pyrethroid (SP) Cypermethrin was bought for the experiments. Every chemical compound was 98% pure and of analytical grade. Two pyrethroid stock solutions (400 mg/L) were made in D/W and filtered by membrane filtration with a pore size of 0.4 µm. The following ingredients made up the mineral salt medium (MSM), which was utilized for enrichment and degradation investigations (in g/L): The final pH was adjusted to 6.8 to 7.0 after adding, 1.5 (g/L) K_2HPO4, 0.5 (g/L) KH_2PO4, 1.0 (g/L) NaCl, 0.5 (g/L), $(NH_2)SO_4$ 0.005, 0.2(g/L) MgSO4.7H2O., (500mg/L) Na_2EDTA, $2H_2O$, (143mg/L)$FeCl_2 .4H_2O$, (4.7mg/L) $ZnCl_2$, (30mg/L)H_3BO_4, (1mg/L)$CaCl_2$, (2mg/L) $NiCl_2.6H_2O$, (1.5%) Agar agar was added at the end (Uzmaa et al., 2023)

2.1. Isolation and enrichment of Pyrethroids degrading bacteria

Pyrethroid-degrading bacteria were isolated using a traditional enrichment procedure. Cypermethrin was added to sterile MSM broth as the only carbon source for enrichment, with a final concentration of 100 mg/L (Uzma et al., 2023). The agricultural land in Dindori Tal. Dist. Nashik, Maharashtra, provided the soil samples, which were collected at a depth of 4-6 cm. A flask with 50 mL of media and 1 gram of soil was shaken at 150 rpm and 30℃ for five days. For an additional five days, five millilitres (5 mL) of the incubated culture were subcultured into fifty millilitres of new MSM media. Seven further iterations of the procedure were conducted using media containing varying doses of Cypermethrin (100, 150, 200, 250, 300, 350, and 400 mg/L). After sufficient growth, the culture was enriched, and then it was serially diluted and spread on MSM agar plates containing 50 mg/L Cypermethrin to isolate individual colonies. Following that, the bacterial colonies were observed and isolated, and then a few organisms were allowed to grow on MSM media that had been supplemented with 50 mg/L cypermethrin. A range of pyrethroid concentrations (50–400 mg/mL) were tested for tolerance in distinct bacterial colonies, and those with a high level of tolerance were chosen for additional examination (Chen, Hu, 2011).

2.2. Morphological and biochemical characterization for identification of bacteria

The physical appearance of the bacterial isolates on culture plates, motility tests, Gram staining, capsule staining by Maneval's method, spore staining by ZNCF method, and other methods were used. The colony morphology and biochemical analysis of the isolates were also performed (Ali et al., 2022; Holt et al., 1994). IMViC tests that is Indole Production test, Methyl red test, Voges Proskauer test and starch hydrolysis test, nitrate reduction test, triple sugar iron agar test, catalase test, oxidase test, sugar fermentation, Gelatin liquefaction and citrate utilization were among the tests employed in biochemical analysis (Ananthnarayan and Paniker, 2020).

Indole Production test. To determine the indole production, a few ml of Kovac's reagent was added to the tube that was pre-inoculated with the organisms in peptone broth. The appearance of a reddish color ('cherry red ring') in the tube on top of the medium within a few seconds of the addition of the reagent indicates a positive test. If the reagent layer remains yellowish or cloudy, the layer shows a negative test.

Methyl red Test. Glucose Phosphate broth was made and sterilised. Following the inoculation of the test organism, the tubes were incubated overnight. After adding the methyl red indicator to the tubes, the colour change was noticed.

Voges Proskauer: The end product of glucose metabolism by various bacteria may be different, even though the majority of bacteria can ferment (metabolize) glucose. Some use the butylene glycol pathway to synthesize products like acetylmethylcarbinol, that is, acetoin and butanediol after glycolysis, while others use the mixed acid fermentation pathway to transform pyruvate into a stable organic acid combination. 24 hours old culture suspension was inoculated in VP broth and after incubation, 6 drops of alpha naphthol in ethanol and 2 drops of 40% Potassium Hydroxide was added. The formation of a pink colour after vigorous shaking indicates a positive test.

Starch hydrolysis. The selected bacterium was added to a sterile starch agar plate, which was then cultured for 48 hours at 28℃. On the plate, 1-2 ml of Gram's Iodine was dripped all over the agar surface. The inoculum was surrounded by a clear zone. A comparison between the inoculated and uninoculated areas was made, and the result was noted.

Nitrate Reduction test: After inoculating nitrate broths with bacterial suspension, incubate the tubes overnight at the ideal temperature of 28°C. Before the addition of the reagents, check for Nitrogen gas. Then, add 6-8 drops of Nitrite reagent A and 6-8 drops of nitrite reagent B. Watch for a change in colour within a minute.

Triple sugar iron test: The 24 hrs. old culture of the test bacterium using a sterile inoculating wire on the slant, and the butt using the inoculating wire was streaked and while withdrawing, streaked slant was stabbed up to 3 to 5 mm above the base of the test tube then incubated at 28℃ for 24 hours. Additionally, within 24 hours of incubation, note the colour change of the butt and slant.

Catalase test. After 24 hours, the isolated culture was taken and put on a slide. On the microscopic slide, a single drop of hydrogen peroxide solution was applied to the culture organism. There were air bubbles visible on the slide. The presence of air bubbles indicates a successful outcome, indicating the ability of the organism to produce catalase enzyme.

Oxidase test: 24 hours old culture was rubbed on Whatmann's filter paper dipped in Oxidase reagent (tetra-methyl-p-phenylenediamine dihydrochloride solution). Observe the change in color. When a microorganism turns dark purple in 5-10 seconds, it indicates the production of the oxidase enzyme. When the colour turns purple in 1-2 mins. Indicates that the microorganism is delayed oxidase positive. If the colour does not change or if it takes more than 2 minutes, the microorganisms cannot synthesize Oxidase, and they are oxidase negative.

Sugar fermentation: The capacity of various bacteria to ferment particular carbohydrates varies; some are able to ferment a single type of carbohydrate, while others are unable to do so. The final results of digesting carbohydrates are organic acids. The medium's pH will drop as a result of these generated acids. The medium's pH indicator changes colour as a result of this pH drop. The inverted Durham tube or fermentation tube is immersed in the fermentation broth and will capture any gases released by the bacteria during the fermentation of carbohydrates as an air bubble. This color change indicates that fermentation has occurred, and the formation of gas bubbles in Durham's tube indicates the release of gas and, hence, confirms that the test bacteria were able to use the selected carbohydrate used in the test medium.

*Gelatin liquefaction*Test bacteria that have been incubated for 24 hours are stab-inoculated into tubes that contain nutrient gelatine media. After 48 hours, the inoculated tubes and an uninoculated control tube are examined for gelatine liquefaction. They are incubated at 30°C. After that, the tubes are submerged for 15 to 30 minutes in an ice bath. Tubes are then tilted to check for hydrolysis of the gelatine. The uninoculated control medium will stay solid, whereas hydrolysed gelatine will produce a liquid medium even after being exposed to cold temperatures (ice bath).

Citrate utilization: Kosser's Citrate broth was inoculated, and the ability of an organism to utilize the Sodium Citrate as the only source of carbon was tested after 24 hours in the form of turbidity in test tubes.

2.3. Growth kinetics

By inoculating each bacterial isolate into 50 ml of medium supplemented with 100 mg/L of cypermethrin, the growth kinetics study of all the organisms isolated (Five) cultured on Luria Bertani agar were assessed. The growth curve (absorbance) of the strains was measured at 620 nm at various intervals throughout their 28°C incubation (5, 20, 40, 60, 80, and 96 hours). The strains with the best growth log phase were chosen based on the medium without cypermethrin, which served as a negative control (Uzmaa et al., 2023).

2.4. Optimization study for the degradation of Cypermethrin (Inoculum size, Temperature and pH)

As *Bacillus stercoris* (PRSB-1) has shown better efficiency of Cypermethrin degradation, it was selected for future study. In order to know the optimum inoculum size of the isolated *Bacillus stercoris*, the organism was grown in the medium containing 100 ppm. Cypermethrin 50 ml of mineral salt medium (MSM) (pH 7.0) was supplemented with cypermethrin at concentration 100 mg l^{-1} (performed in three sets). 10^6 bacterial cells per ml were inoculated in different quantities such as 1 ml, 2ml, 3ml, 4ml and 5ml. The organisms were then incubated at room temperature near about 28°C (pH 7) at 180 rpm for 120 hours (5 days) on a rotary shaker. The bacterial cell density was measured by studying the O.D. at 620 nm every 24 hours in a spectrophotometer.

The optimum temperature determination is performed by inoculating the 24-hour-old culture in a flask containing 25 ml of sterile MSM broth containing 100mg/L Cypermethrin concentration. The flasks were incubated at different temperatures, such as 20°C, 25°C, 30°C, 35°C, 40°C, 45°C, and 50°C for 48 hours. The absorbance was noted down after incubation, and the uninoculated flask was kept as blank.

A range of pH values (4.5, 5.0, 6.0, 7.0, 8,0, 9.0, and 10.0) were achieved by setting up different sample aliquots of 50 mL MSM containing Cypermethrin and then inoculating them with the isolated bacteria. Using a spectrophotometer, the growth of cultures was observed for 48 hours at 37°C and was studied at 620 nm. By charting the absorbance readings and pH, the ideal pH was ascertained, with the negative control being kept as blank.

2.5. Molecular analysis

Identification carried out at NCMR

Bacterial strains are identified. The National Centre for Microbial Resource (NCMR) sequencing facility at the National Centre for Cell Science in Pune was used to identify the isolates. Genomic DNA was isolated at the facility using the conventional phenol/chloroform extraction procedure (Sambrook J, Fritsch, 1989). Using universal primers 16F27 [5'-CCA GAG TTT GAT CMT GGC TCA G-3'] and 16R1492 [5'-TAC GGY TAC CTT GTT ACG ACT T-3'], the 16S rRNA gene was subsequently amplified by PCR. Following the manufacturer's instructions, the amplified 16S rRNA gene PCR product was directly sequenced using an ABI® 3730XL automated DNA sequencer (Applied Biosystems, Inc., Foster City, CA) after being purified by PEG-NaCl precipitation. To ensure that every position was read at least twice, sequencing was essentially done from both ends using extra internal primers. The EzBioCloud database was used for identification after the Lasergene package was used for assembly (Oros, D.R. and Werner, I., 2005).

General sequencing protocol

PCR amplification employing primers for the 16S rRNA region after DNA isolation (PCR Template preparation) using the Phenol-Chloroform technique. Check the agarose gel's amplification. PEG-NaCl PCR purification → primer-based cycle sequencing → cycle sequencing cleanup → Samples were loaded into the ABI® 3730 XL.

The 16srRNA sequence is submitted to NCBI databases to obtain the accession number.

2.6. Determination of degradation potential

The bacterial isolate was inoculated in various concentrations of pesticide. The inoculum size was adjusted to O.D. 0.8. The O.D. was measured at 290nm, and the difference in the OD was recorded. 1 ml of bacterial (*Bacillus stercoris*) culture was transferred to 50-ml MSM containing 50 mg l^{-1}, 100 mg l^{-1}, 150 mg l^{-1}, 200 mg $l^{-1,}$ and 250 mg l^{-1}cypermethrin and was allowed to grow for 7 days at 40 °C under agitating conditions in a shaker incubator at 150 rpm. Samples were withdrawn at the intervals of 24 hours, and absorbance was observed.

The determination of degradation potential was calculated using the following formula.

Degradation percentage calculation

$$\textbf{Degradation (\%)} = \left[\frac{C0-Ct}{C0}\right] \times 100$$

Where, C0 = Initial concentration of Pesticide

Ct = Concentration after time t

2.7 Detection of metabolites after GC-MS of biodegraded Cypermethrin

The analysis was carried out at CIF, SPPU, Pune, and the metabolites were identified. GC, which is based on liquid-liquid partitioning with acetonitrile, followed by a cleanup step with dispersive-SPE (Solid Phase Extraction). The GC-MS chromatogram of cypermethrin with

strain PRSB-1 is shown in Figure 3.7. Metabolites were identified by comparison with the GC-MS compound library NIST 14.lib. (Table 5). The sample preparation was done by the standard method mentioned in a research paper by Pankaj Bhat et al. (2020).

Results:

3.1 Soil samples collected from pesticide-containing agricultural fields were processed for isolation, identification, and characterization of SPs-degrading bacteria. Five SPs-degrading bacteria were isolated through a conventional enrichment culture technique using MSM supplemented with cypermethrin as the sole carbon source. All the bacterial strains showed growth even at higher concentrations on SPs-supplemented MSM, indicating the capability of strains to utilize cypermethrin as a C source. The growth kinetics of all five strains varied from each other. However, PRSB-1 showed the highest growth on 200 mg/L cypermethrin as compared to other strains.

3.2 Biochemical characterization

The morphological and biochemical characterizations of the isolated organisms are summarized in Table 1. Out of all the five isolates, two isolates were found to be Gram-negative and three Gram positive, four non-spores forming and one spore former mostly rod-shaped bacteria. All were non capsulated and non-motile except PRS-1. Gelatin liquefaction was shown by only one organism (PRSB-1). Nitrate reduction was observed in three cultures. Three strains, PRS-1, PRS-2, and PRSB-2, were unable to produce Catalase, Oxidase, and Indole. PRS-3 and PRSB-3 were biochemically identical. Three appeared positive for starch hydrolysis and citrate utilization, four for Methyl-red, and all have shown Voges Proskauer test negative. Lactose, sucrose, and Maltose fermentation and Triple Sugar Iron tests were studied, and conclusions were made about the probable genus of the isolated strains. All these observations helped to figure out the possibility of degrading organisms from different genera. Bergey's Manual of Systematic Bacteriology was studied to determine the genera. Based on the data available, PRS-1, PRS-2, PRS-3, PRSB-1, and PRSB-2 strains were identified as members of genus *Pseudomonas, Klebsiella, Bacillus, Bacillus,* and *Lactobacillus* species, respectively.

Table 1. Physio-biochemical characteristics of the isolated bacterial strains from agricultural soil.

Characteristics	**Isolated bacterial strains**				
Culture code	**PRS-1**	**PRS-2**	**PRS-3**	**PRSB-1**	**PRSB-2**
Colony Size	3mm	1mm	3mm	1mm	3mm
Colony Shape	Circular	Circular	Irregular	Circular	Circular
Gram stain	Gram Negative	Gram Negative	Gram Positive	Gram Positive	Gram Positive
Cell Morphology	Rods	Short rods	Coccobacilli	Rods	Rods
Spore stain	Non spore former	Non spore former	Non spore former	Spore forming	Non spore former
Capsule stain	Non capsulated	Non capsulated	Non capsulated	Non capsulated	Non capsulated
Motility	Motile	Non-Motile	Non-Motile	Non-Motile	Non-Motile
Gelatin liquefaction	No liquefaction	No liquefaction	No liquefaction	Liquefaction observed	No liquefaction
Nitrate reduction	No	No	Yes	Yes	Yes

Catalase		Negative	Negative	Positive	Positive	Negative
Oxidase		Negative	Negative	Positive	Positive	Negative
Indole production		Negative	Negative	Positive	Positive	Negative
Methyl Red		Negative	Positive	Positive	Positive	Positive
Voges-Proskauer		Negative	Negative	Negative	Negative	Negative
Citrate utility		Negative	Positive	Positive	Positive	Negative
Starch Hydrolysis		Negative	Negative	Positive	Positive	Positive
Lactose fermentation		No Acid, No Gas	No Acid, No Gas	Only Acid, No Gas	Only Acid, No Gas	Acid and Gas production
Sucrose fermentation		No Acid, No Gas	No Acid, No Gas	No Acid, No Gas	Only Acid, No Gas	Acid and Gas production
Maltose fermentation		No Acid, No Gas	No Acid, No Gas	Only Acid, No Gas	Only Acid, No Gas	Acid and Gas production
TSI test	**Slant**	K	K	K	A	K
	Butt	K	A	A	A	A
	H_2S	Negative	Positive	Negative	Negative	Negative
	Gas	Negative	Negative	Negative	Negative	Negative
Probable Identification		*Pseudomonas spp.*	*Klebsiella Spp.*	*Bacillus Spp.*	*Bacillus Spp.*	*Lactobacillus spp.*

3.3. Growth kinetics

From the results of growth kinetic studies, bacterial strain PRSB-1, that is *Bacillus stercoris*, was selected as the best organism for the biodegradation of Cypermethrin, and its optimum inoculum size, temperature, and pH were determined (table 2, fig. 3.1).

Table 2: Growth kinetics study for all five strains

	Growth Absorbance (620nm)				
Tme intervals (Hours)	**PRS-1 (*Pseudomonas Spp.*)**	**PRS-2 (*Klebsiella spp.*)**	**PRS-3 (*Bacillus spp.*)**	**PRSB-1 (*Bacillus spp.*)**	**PRSB-2 (*Lactobacillus spp.*)**
5	0.04	0.05	0.04	0.05	0.04
24	0.47	0.53	0.62	0.54	0.67
48	0.96	0.78	0.98	0.59	0.97
72	1.58	1.43	1.76	1.98	1.56
96	1.19	1.28	1.23	1.67	0.78
120	0.65	0.56	0.45	0.87	0.68

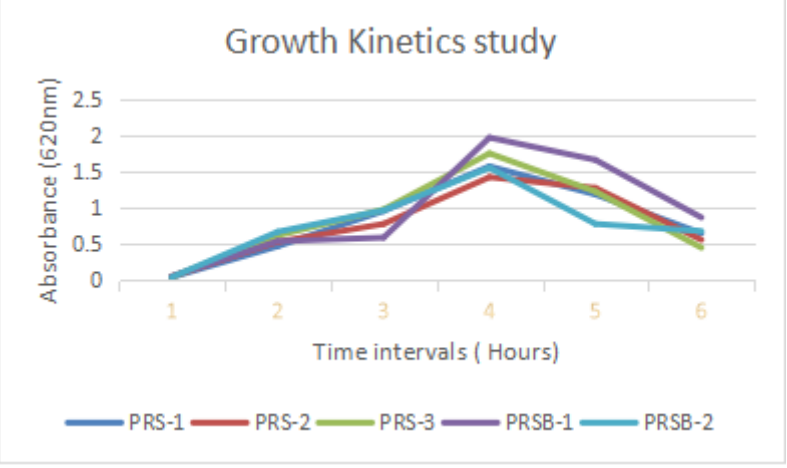

Fig. 3.1 Growth kinetics study

3.4. Inoculum size, Temperature, and pH optimization

It was observed that the inoculum size also affects the growth in pesticide-containing media. It is clearly seen that the turbidity is found more when the cell population was more (3.2). Figures 3.3 and 3.4 provide a summary of the outcomes of optimising the temperature and pH of *Bacillus stercoris* in MSM containing 100 mg/L cypermethrin, respectively. After 48 hours of incubation, *Bacillus stercoris* showed the greatest growth at 40°C in both MSM enriched with cypermethrin (Figure 2.1). Following the 40°C, the log phase of *Bacillus stercoris* growth decreased, with the greatest bacterial growth fall occurring at 55°C. Similarly, after 48 hours of incubation at 40°C, the ideal pH for strain *Bacillus stercoris* was found to be 7 (Figure 3.4). After 48 hours of continuous incubation at pH 3 and pH 4, the least amount of bacterial growth was seen; however, at pH 8, 9, and 10, the amount of bacterial growth against cypermethrin abruptly decreased.

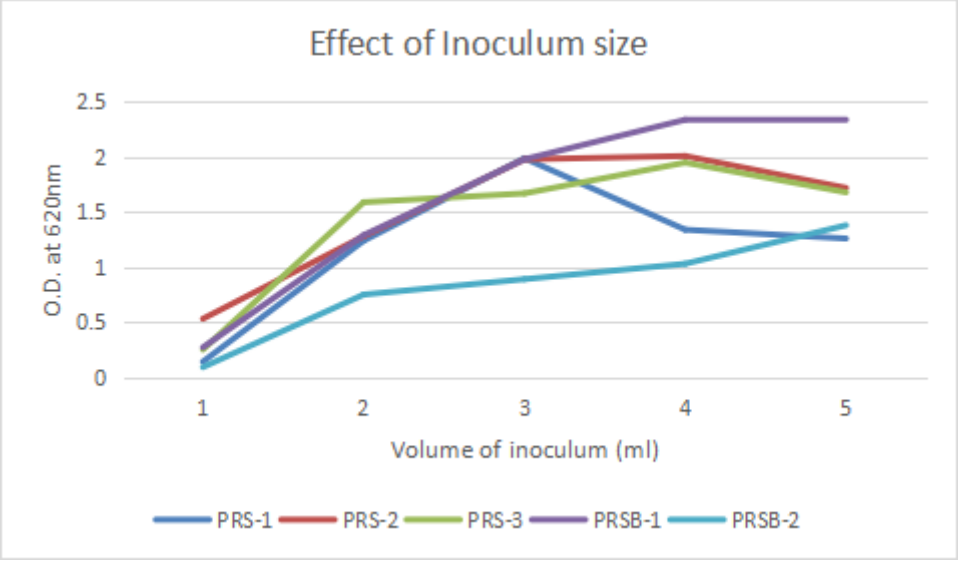

Fig 3.2 Effect of inoculum size

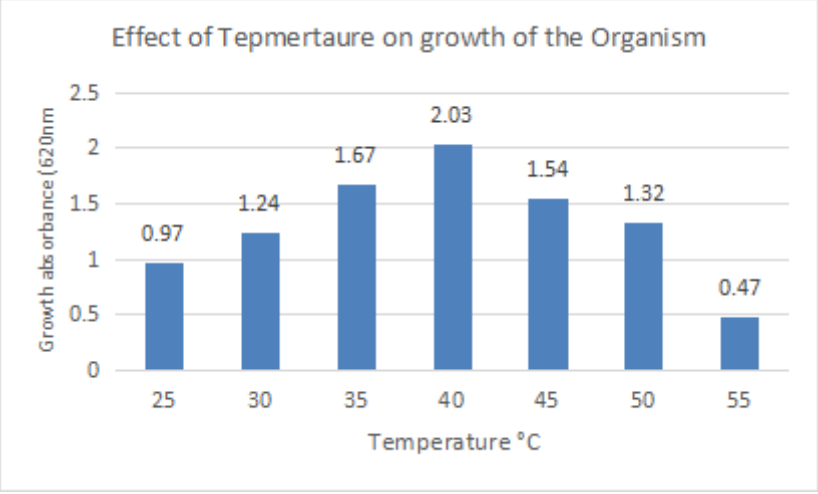

Fig. 3.3 Effect of Temperature

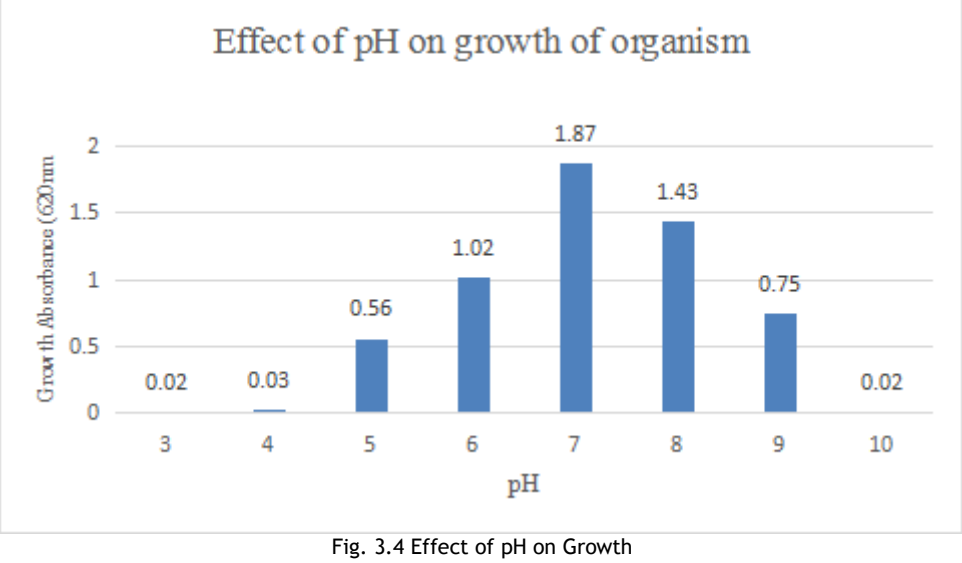

Fig. 3.4 Effect of pH on Growth

3.5. Molecular analysis

After 16S rRNA gene nucleotide sequences were retrieved, they were verified using MEGA-11 program to create a phylogenetic tree and blasting at NCBI databases. According to the strain's phylogenic study, it is 100% comparable to *Bacillus stercoris* JCM 30051(T), which has accession number MN536904. Under the accession codes SUB14193329 PP239385, the nucleotide sequences were uploaded to the NCBI GenBank database (Figure 3.5).

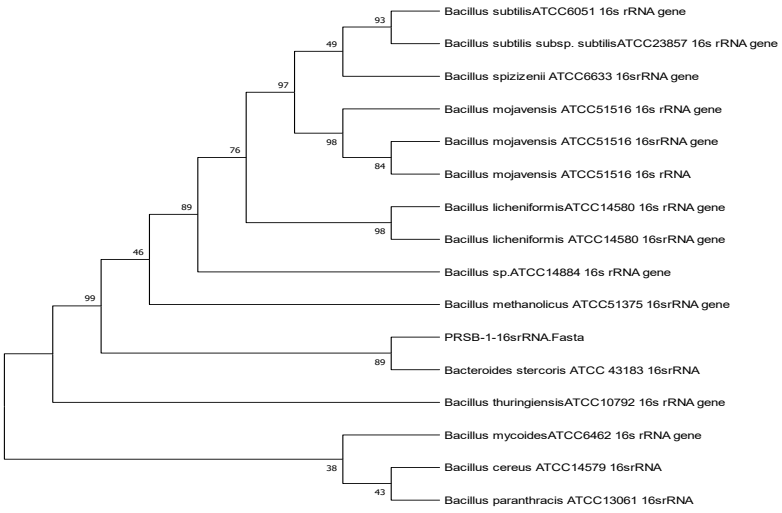

Fig. 3.5 Phylogenic tree of *Bacillus stercoris*

3.6 Determination of degradation potential:

Bacteria was allowed to grow in various concentrations of Cypermethrin and has shown potential to tolerate the high concentration of pesticide like 200mg l^{-1} (fig 3.6).

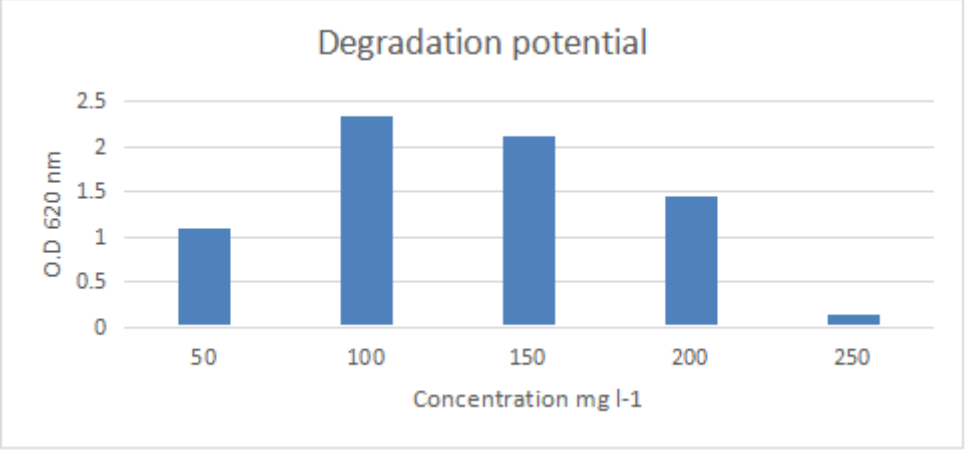

Fig 3.6 Degradation potential study

3.7 GC MS of biodegraded Cypermethrin

The GC MS analysis (fig. 3.7) has shown that Cypermethrin was metabolized by the microorganism, and the products obtained are smaller compounds with low molecular weight. The metabolites were detected at retention time 7.705 minutes and identified as Heneicosane, as 1-Docosanol, acetate and Squalene was identified at retention time 9.701 and 13.392 minutes respectively. Table 5 describes the details of the products obtained after degradation. Yet, a few products were not completely identified, but further study can help to identify those metabolites, too. Metabolic product analysis verified that strain PRSB-1-based degradation did not result in the production of any hazardous intermediates. As a result, this strain's metabolic pathway can efficiently and break down cypermethrin.

Event Time :0.300sec
Scan Speed 3333
Start m/z :40.00
End m/z :800.00
Q1 Resolution : -
Q3 Resolution : -

Sample Inlet Unit :GC

[MS Program]
Use MS Program :OFF

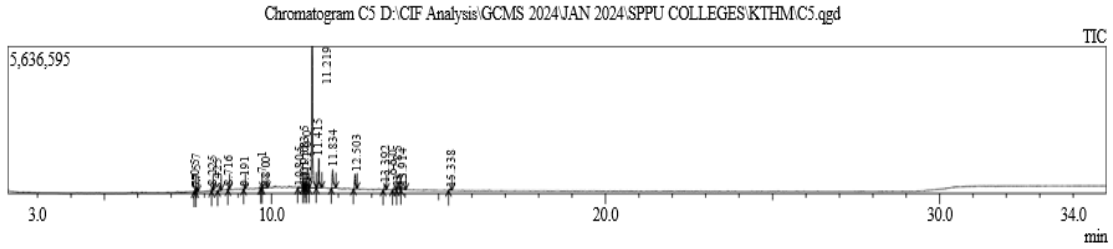

Fig 3.7 Degradation products of Cypermethrin identified by GC MS

Table 5: Degradation products of Cypermethrin identified by GC MS (Peak Report of TIC)

Peak#	R.Time	Area	Area%	Height	Height%	Name
1	7.705	132617	0.68	71648	0.75	Heneicosane
2	7.757	137056	0.70	72340	1.76	Hexadecane, 2,6,10,14-tetramethyl-
3	8.225	144304	0.74	105745	11.11	Dotriacontane
4	8.425	156049	0.80	48333	0.51	1,2-Benzenedicarboxylic acid, bis(8-methylno
5	8.716	147106	0.76	123885	11.30	Eicosane
6	9.191	110723	0.57	86095	1.79	Tetracosane
7	9.701	154370	0.79	99622	3.67	1-Docosanol, acetate
9	10.805	173950	0.89	57061	3.36	
10	10.958	269691	1.39	164604	11.73	
11	11.035	368038	1.89	170321	7.79	
12	11.100	346047	1.78	101321	20.26	
13	12.503	1236354	6.36	563837	5.93	4-t-Butyl-2-(4-methoxy-phenyl)-6-p-tolyl-pyri
14	13.392	536435	2.76	208288	2.19	Squalene
15	13.660	330318	1.70	103321	1.09	Cypermethrin
16	13.785	269965	1.39	92391	0.97	Cypermethrin
17	13.914	384689	1.98	75348	0.79	Cypermethrin
18		19449473	100.00	9506537	100.00	

DISCUSION

Up until 1985, cypermethrin was widely utilised in home and agricultural settings. It was found to have an impact on numerous vertebrate systems, necessitating more research. Since 2006, the European Union has authorised cypermethrin, a common pyrethroid insecticide used to control agricultural pests. Citing the European Food Safety Authority's (EFSA) finding of possible hazards to aquatic life and honeybees, PAN Europe challenged the Commission's decision to renew.

In the present study, the bacteria were isolated from Cypermethrin contaminated soil and were identified with the help of biochemical and molecular characterization. PRSB-1 was characterized as *Bacillus stercoris* ATCC 431183 (Accession No. SUB14193329 PP239385). This organism has shown the efficient degradation of Cypermethrin and the products obtained were completely non toxic for the environment. Along with this, the other organisms identified up to genus level were *Pseudomonas, Klebsiella, Bacillus,* and *Lactobacillus* species.

All these organisms have also shown the degradation up to a specific level, but their optimization study would help to determine the degradation potential. Inoculum size, optimum temperature, and optimum pH are the important parameters for the degradation of pesticides. The optimum conditions can help the organisms to metabolize the pesticide and use it as a source of carbon. These results were consistent with findings reported by Chen et al. (2012c), who found that ß-cypermethrin bio-degradation likely occurs in neutral and alkaline conditions.

Following a 48-hour peak in cell biomass across all treatments, a decline in growth was noted. For both bacterial strains, the maximum biomass was observed at 100 mg l^{-1}Cypermethrin concentrations, and the lowest for control samples was 0mg l^{-1}. Another significant issue at the application level is the inoculum's size. The bacterial cells were injected at a rate of one milliliter per 10^8 cells ml^{-1} in 50 millilitres of mineral salt medium (MSM) for each of the aforementioned studies. Research has demonstrated that when inoculated at concentrations of 10^6-

10^{10} cfu ml^{-1}, microorganisms that can break down pyrethroids show an increased rate of degradation and a shorter half-life of the pesticide.

A key factor in regulating the breakdown of pyrethroids is temperature. in line with the strain's development. Growth must be at its highest levels for deterioration to be at its maximum, which requires ideal growth conditions. Zhang et al., (2016) conducted temperature-dependent degradation investigations, which demonstrated that the pyrethroid degradation process is negatively impacted by both high and low temperatures.

Our findings also demonstrated that cypermethrin degraded most readily at pH 7.0. For an organism to be utilised for bioremediation in a variety of settings, this is a crucial characteristic. The optimum pH being at about 7 indicates that under extreme acidic pH such as 3 and 4, degradation was completely absent so such conditions might stabilize Cypermethrin and same stability was observed at pH 10 too. At pH 6 and 8, degradation rates were insignificant. Cypermethrin removal by *Bacillus* strain PRSB-1 was highest at pH 7, followed by 8 and then 6. Below pH 6 and above pH 8, the degradation rate was not appreciable.

The study of degradation potential helps us to understand the ability of the organism to use the pesticide as the sole source of Carbon.

Cypermethrin and its intermediate metabolites were effectively detected and characterized by GC MS analysis to determine the metabolic products of Cypermethrin. Products like Heneicosane, Hexadecane, Dotriacontane, Eicosane, Tetracosane, and 1-Docosanol, acetate were detected after GC MS. It was studied that these products are not toxic to the environment and many are beneficial with the role as antiviral agents, antioxidants etc.

Numerous studies have examined the negative consequences of pesticide use and how it affects the ecosystem. While pyrethroids are somewhat less harmful to non-target organisms, prolonged exposure to them can harm organ functions (Soni et al., 2011) and have anti-androgenic and carcinogenic effects (Zhang et al., 2010).

Consequently, it is crucial to clean up pesticide-contaminated areas and stop additional buildup. The necessity for a safe, effective, and cost-effective method of removing pesticides from contaminated environments is highlighted by the established harm that pyrethroids cause to the ecosystem. This research work makes a minor addition to the larger picture of bacteria-mediated bioremediation techniques.

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