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Original Article

Isolation and characterization of synthetic pyrethroids-degrading bacterial strains from agricultural soil

Isolamento e caracterização de cepas bacterianas degradadoras de piretróides sintéticos de solo agrícola

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Abstract

Pyrethroid pesticides are commonly used for pest control in agriculture setup, veterinary and home garden. They are now posing increased risks to non-targeted organisms associated to human beings due to their considerable use. The present work deals with the isolation of bacteria with tolerance to high concentrations of bifenthrin and cypermethrin from contaminated soil. Enrichment culture technique (bifenthrin concentration = 50-800 mg/L) was used for bacterial isolation. Bacteria that showed growth on minimal media with bifenthrin were also sub-cultured on minimal media with cypermethrin. Bacteria showing luxurious growth on both the pyrethroid, were screened out based on their morphological, biochemical parameters and by API 20NE Kit. Phylogenetic studies revealed that, one bacterial isolate (MG04) belonging to *Acinetobacter lwoffii* and other five bacterial isolates (MG06, MG05, MG01, MG03 and MG02) cluster with *Pseudomonas aeruginosa*, *Pseudomonas putida* respectively. Isolated members of genera *Pseudomonas* and *Acinetobacter* could be used for further detailed degradation studies by using FTIR, HPLC-MS or GC-MS analysis.

Keywords: synthetic pyrethroids degradation, soil bacterial *spp*., *Pseudomonas spp*., *Acinetobacter spp*.

Resumo

Os pesticidas piretróides são comumente usados para controle de pragas na agricultura, veterinária e hortas domésticas. Atualmente eles apresentam riscos aumentados para organismos não-alvo associados a seres humanos devido ao seu uso considerável. O presente trabalho analisou o isolamento de bactérias com tolerância a altas concentrações de bifentrina e cipermetrina de solo contaminado. A técnica de cultura de enriquecimento (concentração de bifentrina = 50-800 mg/L) foi utilizada para o isolamento bacteriano. Bactérias que apresentaram crescimento em meio mínimo com bifentrina também foram subcultivadas em meio mínimo com cipermetrina. Bactérias apresentando crescimento luxuoso em ambos os piretróides foram triadas com base em seus parâmetros morfológicos, bioquímicos e pelo Kit API 20NE. Estudos filogenéticos revelaram que, um isolado bacteriano (MG04) pertencente a *Acinetobacter lwoffii* e outros cinco isolados bacterianos (MG06, MG05, MG01, MG03 e MG02) agrupam-se com *Pseudomonas aeruginosa, Pseudomonas putida* respectivamente. Membros isolados dos gêneros *Pseudomonas e Acinetobacter* podem ser usados para estudos de degradação mais detalhados usando análises de FTIR, HPLC-MS ou GC-MS.

Palavras-chave: degradação de piretróides sintéticos, espécies bacterianas do solo, *Pseudomonas spp*., *Acinetobacter spp*.

1. Introduction

Synthetic pyrethroids (SPs) are the major substitute of organophosphates (OPs), which have been of current research interest due to high toxicity and recalcitrant characteristics (Oros and Werner, 2005). Currently, pyrethroids account for more than 25% of commercially used insecticides worldwide (Zhang et al., 2010). Pyrethroids contain type-I and type-II groups on the basis of chemical structure and toxicological actions. For example, type-II contain an α-cyano group in their chemical structures while type-I do not (Laskowski, 2002).

More recently, pyrethroids have been widely used for pest control more importantly in studies have found confirmed health issues associated with ingestion of pyrethroids as its use enormously increased public health

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concerns and put people at risk of various diseases and conditions such as damage to endocrine, reproductive and immune system and increased chances of cancer (ATSDR, 2003; Zhang et al., 2010). Pyrethroids also cause environmental issues like air, soil and water pollution (Cycoń et al., 2006; Decourtye et al., 2005; Wendt-Rasch et al., 2003). SPs are enormously toxic to aquatic life, even only 10 ng/L concentration is enough to eradicate the entire invertebrate population of a river or lake (Pearce and Warford, 2012). The SPs pollution occurs through treated livestock in dipping tanks and/or directly from agricultural soil run off (Armstrong and Phillips, 1998). In Pakistan, increased than standard (European Union Standard for Drinking Water) pyrethroid residues have found in Rawal and Simly Dam Lakes, Islamabad (Iram et al., 2009). One of local newspaper also reported high fish mortality in these lakes that is probably due to excessive use of SPs in agricultural fields of National Agriculture Research Council (NARC) which are adjacent to these lakes.

Nowadays photolysis, hydrolysis and biodegradation are the commonly used approaches for disposal of SPs in agricultural lands (Fan et al., 2012). Biodegradation is the most suitable method for the disposal of pesticides residues and is accomplished by microbial forging activities. However, rate of biodegradation largely depends on type of microbial community composition and diversity, type of soil and pesticide and climatic conditions as well. Important to note is that microbial degradation of SPs is also highly environmental friendly, effective, cheap and safe practice to clean up these environmental contaminants (Singh and Walker, 2006). Unfortunately, biodegradation approach is still not in practices to detoxify these environmental pollutants in Pakistan. Thus, it is necessary to develop rapid and effective methods to remove these toxic SPs constituents that may reduce the environmental and public health risks associated with pyrethroid usage. Biodegradation is a major practical and applicable approach for the detoxification of SPs in various conditions such as either in soil or in aquatic environment.

To date, many researches have done on isolation and evaluation of pyrethroid degrading bacteria and fungi (Cycoń and Piotrowska-Seget, 2016; Fan et al., 2012; Zhang et al., 2010). However, previous studies on biodegradation of SPs used microbes that could only use carbon as sole energy source (Grant et al., 2002), and these conditions may not be suitable for biodegradation in open environment.

Also, bifenthrin a type-I pyrethroid (Hintzen et al., 2009) which is classified as toxicity class-II moderately hazardous compound (WHO, 2009) is the most persistent and obstinate pyrethroid to microbial degradation (Wang et al., 2009). Only few studies have reported bifenthrin degrading microbes (Chen et al., 2012). Similarly, cypermethrin — a type-II pyrethroid has become one of the dominant insecticides among retail sales to consumers (Weston et al., 2009) and regarded as a possible human carcinogen by the Environmental Protection Agency (EPA) USA (Zhang et al., 2010). So, there is a strong need to develop effective strategies for the removal of SPs residues due to their high toxic effects on mankind and environment. The present study was aimed to isolate and identify the bacterial strain from agricultural soil with degradation and tolerance potential to higher concentrations of bifenthrin and cypermethrin using different optimizing conditions.

2. Materials and Methods

2.1. Chemicals and media

Synthetic pyrethroids (SPs) bifenthrin and cypermethrin were purchased from Sigma-Aldrich (USA). All the chemicals were of analytical grade and 98% pure. Stock solutions of both the pyrethroids (1000 mg/L) were prepared in methanol and filtered by membrane filtration (pore size 0.22 µm). Mineral salt medium (MSM) used for enrichment and degradation studies was composed of the following components (in g/L): 1.0 NH_4 NO_3, 1.0 NaCl, 1.5 K_2 HPO_4, 0.5 KH_2PO_4, 0.005 $FeSO_4 7H_2O$ and 0.2 $MgSO_4.7H_2O$, and final pH was adjusted to 7.0. For solid media, 15 g/L agar was added to mixture of all the media constituents.

2.2. Isolation and enrichment of SPs degrading bacteria

A conventional enrichment technique was performed for isolation of pyrethroids degrading bacteria. Enrichment was carried out in MSM supplemented with bifenthrin (initial concentration 50 mg/L and final concentration 800 mg/L) as an additional carbon source. The soil samples at 4-6 cm depth were collected from the agricultural fields of NARC Islamabad, having five years' history of pyrethroid usage. One-gram soil was transferred to 50 mL media in a flask and incubated for 5 days at 30 °C temperature and 180 rpm in a shaker incubator. Five (5 mL) of the incubated culture was sub-cultured into 50 mL fresh MSM medium for another 5 days. The process was further repeated seven times with media containing bifenthrin at different concentrations (200, 300, 400, 500, 600, 700 and 800 mg/L). After enrichment, the culture was serially diluted and spread on MSM agar plates (supplemented with 50 mg/L bifenthrin) for isolating individual colonies. Bacterial isolates were then cultured on MSM media supplemented with 50 mg/L cypermethrin. Distinct bacterial colonies were checked for tolerance to various pyrethroids concentrations (50 to 800 mg/mL) and those possessing high degree of tolerance were selected for further analysis.

2.3. Phenotypic characterization of bacteria

Colony morphology and biochemical analysis of the bacterial isolates was done through their physical appearance on culture plates, gram-staining, capsule staining, spore staining and motility test (Ali et al., 2022; Holt et al., 1994). Biochemical analysis involved tests such as gelatin liquefaction, starch hydrolysis, triple sugar iron agar test, nitrate reduction, catalase test, oxidase test, methyl red, Voges Proskauer, citrate utilization and fermentation of sugars. Further API (Analytical Profile Index) 20NE, BIOMERIEUX, France was used to confirm the biochemical characterization of isolated bacterial strains.

2.4. Growth kinetics

The growth kinetics of all bacterial isolates grown on LB agar were estimated by inoculating into 50 mL MSM

media supplemented with cypermethrin and bifenthrin each at 800 mg/L concentration. During incubation at 37 °C, the growth curve of strains was measured at 620 nm at different time intervals (4, 20, 40, 60, 80 and 96 hours). The medium without SPs was considered negative control and best strains were selected on the base of growth log phase.

2.5. Temperature and pH optimization

For pH optimization, different aliquots of 50 mL MSM supplemented with SPs were set up at different pH (4.5, 5.0, 6.0, 7.0, 8.,0, 9.0 and 10.0) and inoculated with the isolated bacteria. The growth curve of 40 h at 37 °C incubated cultures was estimated at 620 nm using spectrophotometer (BioRad, USA). Negative control was left blank and optimum pH was determined by plotting absorbance values and pH.

2.6. Genomic DNA isolation

Isolation of genomic DNA was done by CTAB (Cetrimonium bromide) method with some modifications (Wilson, 2001). Bacterial isolates were inoculated on nutrient agar plates and incubated for at 37 °C. Loops (n=3) of the fresh culture were added to 300 µL of TE buffer and vortexed at high speed. Five (5 µL) 20 mg/mL proteinase K and 30 µL of 10% SDS were added, and mixture was incubated at 37 °C for 1 hour in water bath, followed by the addition of 100 µL CTAB solution and 100 µL 5 M NaCl and incubation again at 65 °C for 30 minutes in water bath. After incubation, 500 µL mixture of phenol, chloroform and isoamyl alcohol at 25:24:01 ratio was applied, and solution was subjected to centrifuge for 20 minutes at 12000 rpm. The supernatant was collected in sterile Eppendorf tubes and pellets were discarded. For DNA extraction, 500 µL isopropanol and 200 µL 3 M sodium acetate was added to the supernatant and kept at room temperature for 30 minutes followed by overnight incubation at freezing temperature at 4 °C. The DNA pellet was recovered by centrifuging an overnight freeze supernatant at 10,000 rpm and 4 °C for 15 minutes. Supernatant was discarded and pellets were washed with 200 µL 70% ethanol by centrifugation at 10,000 rpm for 5 minutes. Ethanol was removed by using blotting paper and DNA pellets were dissolved in 50 µL TE buffer for amplification of DNA.

2.7. Molecular analysis

The amplification of 16S rRNA partial gene sequences were performed using previously described method (Sakamoto et al., 2010) (Keratec, Korea). The universal primers U1F (5-CCAGCAGCCGCGGTAATACG-3) and UnR (5-GGACTACCAGGGTATCTAAT-3) were used (Barghouthi, 2011). Fifty (50 µL) PCR master mix used in the study was prepared using 5 µL buffer (10X), 0.7 µL *Taq* polymerase, 2.5 µL $MgCl_2$, 2 µL dNTPs, 1 µL each forward and reverse primer, 2.5 µL DNA and 36.5 µL PCR water.

The thermal profile used for amplification reaction was initial denaturation at 95 °C for 5 minutes, 35 cycles of denaturation at 95 °C for 30 seconds, annealing at 55 °C for 45 seconds, extension at 72 °C for 90 seconds and a final extension for 10 minutes. The hold was set at 4 °C and PCR product was visualized by 1% (w/v) electrophoresis using UV-Transilluminator. Amplified products were purified using the PCR purification kit (Thermo Scientific) and sent for sequencing.

Upon retrieval of nucleotide sequences, they were confirmed through blasting at NCBI and EzBioCloud databases and MEGA-X software to construct phylogenetic tree. The nucleotide sequences were submitted in NCBI GenBank database under the accession numbers OK148706 to OK148710 and MG957215.

3. Results

Soil samples collected from pyrethroid containing agricultural fields were processed for isolation, identification and characterization of SPs-degrading bacteria. Six SPs-degrading bacteria were isolated through conventional enrichment culture technique using MSM supplemented with bifenthrin and cypermethrin pyrethroids as sole carbon source. All the bacterial strains showed growth even at higher concentrations on SPs supplemented MSM, indicating the capability of strains to utilize the bifenthrin and cypermethrin as C source. The growth kinetics of all six strains were varied from each other, however MG-04 showed highest growth on 800 mg/L bifenthrin and cypermethrin as compared to other strains (Figures 1a and b).

The morphological and biochemical characteristics of the isolated organisms are summarized in the (Table 1). All the isolates were gram-negative, non-spore forming, rod shaped bacteria. MG-04 was non-motile and positive for capsule staining, whereas the rest of the isolates were non-capsulated motile bacteria. Three strains MG-03, MG-05, and MG-06 were biochemical identical. They appeared positive for starch hydrolysis, nitrate reduction, catalase, oxidase, urease, indole production, methyl-red, citrate utilization and dextrose fermentation and negative for gelatin liquefaction, Voges-Proskauer and fermentation of lactose, maltose and sucrose. Based on presumptive identification, MG-01, MG-02, MG-03, MG-05 and MG-06 strains were identified as members of genus *Pseudomonas*, however MG-04 was an *Acinetobacter sp*. API 20NE further confirmed this identification.

From the results of growth kinetic studies, bacterial strain *Acinetobacter sp*. MG-04 was selected best candidate for the biodegradation of SPs and determined its temperature and pH optimization. The results of temperature and pH optimization of *Acinetobacter sp.* MG-04 in MSM containing 800 mg/mL bifenthrin and cypermethrin are summarized in Figures 2a and 2b respectively. *Acinetobacter sp.* MG-04 indicated highest growth at 40 °C in both MSM supplemented with bifenthrin and cypermethrin after 40 hours of incubation (Figure 2a). The log phase of MG-04 growth declined after the 40 °C, and highest bacterial growth decline was observed at 55 °C. Likewise, optimum pH for strain MG-04 was determined 7 and 8 after 40 hours' incubation at 40 °C (Figure 2b). The minimum bacterial growth was noticed at 4 pH, however, after 8 pH a drastic decline in bacterial growth

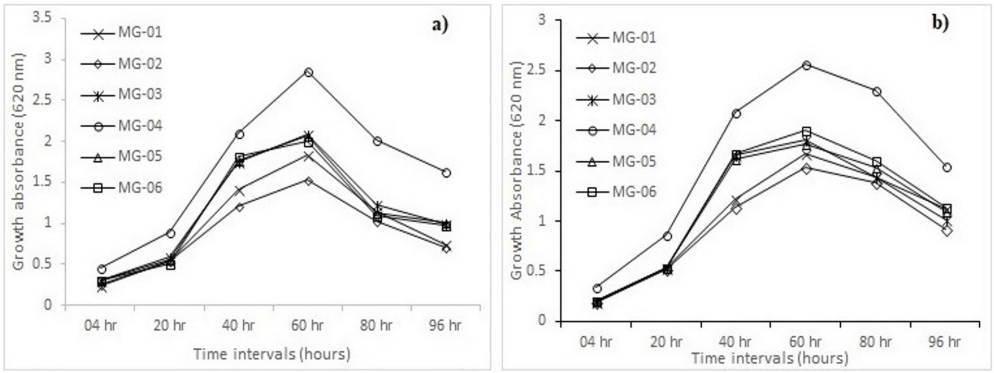

Figure 1. Growth kinetics of six bacterial strains by the degradation of 800 mg/mL (a) Bifenthrin and (b) Cypermethrin supplemented in Mineral Salt Medium (MSM).

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

Characteristics		Isolated bacterial strains					
		MG-01 (OK148710)	**MG-02 (OK148709)**	**MG-03 (OK148708)**	**MG-04 (MG957215)**	**MG-05 (OK148707)**	**MG-06 (OK148706)**
Gram stain		-	-	-	-	-	-
Shape		Rod	Rod	Rod	Rod	Rod	Rod
Spore stain		-	-	-	-	-	-
Capsule stain		-	-	-	+	-	-
Motility		+	+	+	-	+	+
Gelatin liquefaction		+	+	-	-	-	-
Nitrate reduction		+	-	+	+	+	+
Catalase		+	+	+	+	+	+
Oxidase		+	+	+	-	+	+
Urea hydrolysis		+	-	+	-	+	+
Indole production		+	-	+	-	+	+
Methyl Red		+	+	+	-	+	+
Voges-Proskauer		-	-	-	+	-	-
Citrate utility		+	+	+	-	+	+
Lactose		-	-	-	-	-	-
Sucrose		-	-	-	-	-	-
Maltose		-	-	-	-	-	-
TSI test	Slant	K	K	K	-	K	K
	Butt	K	N	K	-	K	K
	H_2S	-	-	-	-	-	-
	Gas	-	-	-	-	-	-
Probable Identification		*Pseudomonas sp.*	*P. putida*	*P. aeruginosa*	*Acinetobacter sp.*	*P. aeruginosa*	*P. aeruginosa*

against bifenthrin and cypermethrin was recorded after 40 hours of continuous incubation.

The NCBI blast of 16S rRNA gene sequence of the best selected strain MG-04 indicated its 98% homology with *Acinetobacter lwoffii* strains. The phylogenetic analysis of this strain indicated its similarity with previously identified *Acinetobacter* strains isolated from different agricultural soils worldwide. Therefore, MG-04 was submitted to GenBank database as *Acinetobacter lwoffii* MG-04 [MG957215] (Figure 3).

The remaining five bacterial isolates (MG-02, MG-06, MG05, MG01 and MG03) of the present study were

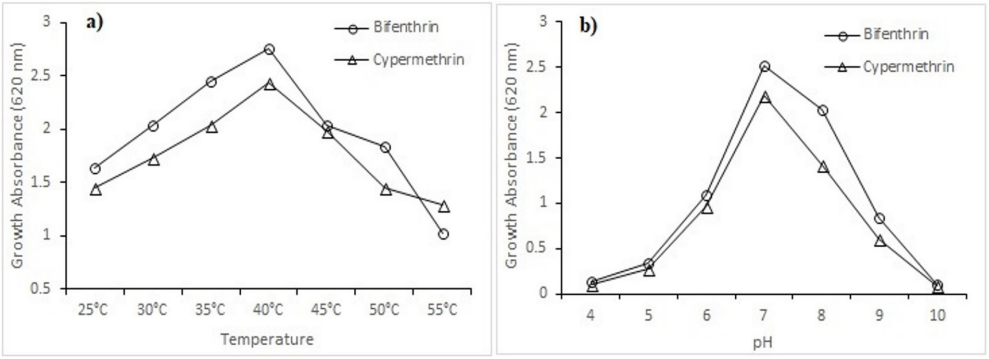

Figure 2. The temperature (a) and pH (b) optimization for the biodegradation capability of bacterial strain *Acinetobacter sp.* MG-04 against the (SPs) Bifenthrin and Cypermethrin's supplemented in Mineral Salt Medium (MSM).

identified as phylogenetically identical to bacteria of the genus *Pseudomonas*. The MG-06 (98%), MG-05 (100%), MG-01 (99%) and MG-03 (99.20%) made clusters with the *Pseudomonas aeruginosa spp.*, while the bacterial isolate MG-02 showed 98% resemblance with the *Pseudomonas putida*. The results were also supported by high bootstrap value along with high similarities with their respective top hit sequences mentioned above (Figure 4).

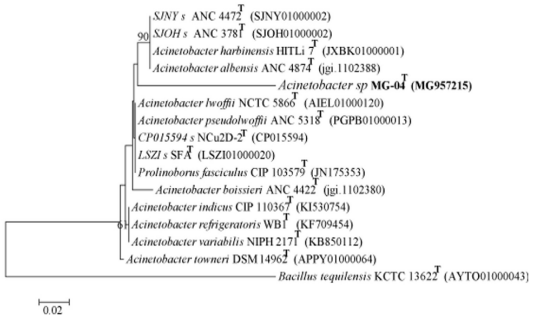

Figure 3. The evolutionary tree was inferred using the Kimura 2-parameter model with bootstrap value (n= 100) was used for computing evolutionary distances of *Acinetobacter lwoffii* MG-04. The optimal tree with the sum of branch length = 0.02 is shown. The tree is drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. Most of the species belonging to *Acinetobacter* genera were used in the tree, whereas *Bacillus tequilensis* KCTC 13622 was used as out group.

4. Discussion

The constant increase in human population, the use of different types of pesticides and fertilizers input gained acceptance in agricultural practices to enhance crops yield (Sabir et al., 2021). Though the literature confirmed that these fertilizers and pesticides enhance crop yield, but studies have also documented unfavorable effects of these fertilizers on soil microorganisms which made these living species adoptable/resistant to surrounding environment (Martelli and Giacomini, 2018; Ryall et al., 2012) similar to antibiotic resistance development in bacteria (Shakeela et al., 2020). Different bacterial and fungal strains have been found resistant to many pesticides (Bhatt et al., 2019), for example, *Candida spp.*, *Aspergillus spp.*, *Pseudomonas spp.*, and *Bacillus spp.*, have been reported as pesticides (including pyrethroids) degrading bacteria (Bhatt et al., 2019; Paul and Mandal, 2019; Singh and Walker, 2006). It has also confirmed that bacteria degrade pyrethroids and use them as carbon source for their growth (Jin et al., 2014).

The current study isolated many bacterial strains from agricultural lands applied with pyrethroid pesticides. Jin et al. (2014) concluded same results from their research. Our study reported that *Acinetobacter spp.*, hydrolyzed pyrethroids and used them as nutrient source for their growth. This is confirmed from previous studies which successfully isolated pyrethroids degrading bacteria (Cycoń and Piotrowska-Seget, 2016; Grant et al., 2002), and their enzymes from soil habitat (Fan et al., 2012).

As mentioned above, the present study isolated *Acinetobacter lwoffii* and *Pseudomonas spp.*, from pyrethroids containing agricultural soil. One of the previous research studies also isolated *Acinetobacter spp.*, from pyrethroids applied soil (Jin et al., 2014), while Gong et al. (2018) isolated *Pseudomonas* species from the same environment. *Acinetobacter lwoffii* and *Pseudomonas spp.*, of the present research have found to have high levels of resistance to bifenthrin and cypermethrin. *Acinetobacter* species are ubiquitous in nature and known to be involved in removal of pesticides such as organophosphate, carbamates and diclofop-methyl herbicides (Bhatt et al., 2021). They are well recognized for remediation of heavy metals (Abdel-El-Haleem, 2003). Previous studies have also shown their role in decontamination of diesel, phenol and crude oil (Jung et al., 2010), but there are very few reports of pyrethroid degradation by members of this genera (Akbar et al., 2015; Jin et al., 2014).

Jin et al. (2014) isolated *Acinetobacter spp.* strain JN8 from soil by enrichment culture technique that could utilize a broad range of only type-II pyrethroids as carbon source for cell growth. Akbar et al. (2015) isolated *Acinetobacter calcoaceticus* MCm5 which degraded both types of pyrethroids. Other previous studies isolated and identified different species of *Acinetobacter* from agricultural soil responsible for degrading pyrethroids and other pesticides (Singh et al., 2004; Singh and Walker, 2006; Chen et al.,

Figure 4. The phylogenetic tree based on 16S rRNA gene of SPs-degrading *Pseudomonas* strain through Neighbor-Joining method. Kimura 2-parameter model with bootstrap value (n= 100) was used for computing evolutionary distances. Most of the species of *Pseudomonas* representing high resemblance with the studied bacterial isolates in different databases were used along with *Acidiferrobacter spp.* 1013-28-CG34 (AY532575) as an out group in the phylogenetic analysis. All positions with <95% site coverage was eliminated.

2012). There is no report of *Acinetobacter lwoffii* having high resistance to pyrethroids earlier. So, this specie is successfully isolated for the first time in the current research effort.

Pseudomonas spp. are an effective bioremediating agents of environmental pollutants such as oil, phenol, azo dyes, organophosphates, organochlorines, carbamates and pyrethroids (Cycoń and Piotrowska-Seget, 2016; Wasi et al., 2013). A previous study (Song et al., 2015) isolated *Pseudomonas aeruginosa* JQ-41 with potential to degrade both bifenthrin and cypermethrin. The newly isolated members of both the genera could have more potential application for treatment of waste from pyrethroid polluted environments after detailed degradation studies and optimization of conditions for degradation. By the use of HPLC, GCMS or other more precise techniques, metabolites produced by bacterial degradation of bifenthrin and cypermethrin could also be identified that will be helpful in designing degradation pathways for pyrethroids by these bacteria. Additionally, bacterial enzymes responsible for degradation of pyrethroids could be purified and applied in similar bioremediation studies in the future.

5. Conclusion

The widespread detrimental impacts of various pesticides on soil ecology and biodiversity due to recalcitrant nature compels the researcher to investigate the biodegradation potential of natural microbial flora of the soil to cope with this threat. The present study was aimed to detect soil microbial candidates with potential to degrade synthetic pyrethroids. *Acinetobacter lwoffii* and *Pseudomonas spp.* were found capable to degrade significant level of degradation of known concentration of synthetic pyrethroids, the optimization of degradation potential was achieved by testing on variable ranges of temperature and pH.

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References

ABDEL-EL-HALEEM, D., 2003. Acinetobacter: environmental and biotechnological applications. *African Journal of Biotechnology*, vol. 2, no. 4, pp. 71-74. http://dx.doi.org/10.5897/AJB2003.000-1014.

AGENCY FOR TOXIC SUBSTANCES AND DISEASE REGISTRY – ATSDR, 2003 [viewed 22 Feberuary 2021]. *Toxicological profile for pyrethrins and pyrethroids* [online]. Atlanta, GA: U.S. Department of Health and Human Services. Available from: https://www.atsdr.cdc.gov/

AKBAR, S., SULTAN, S. and KERTESZ, M., 2015. Bacterial community analysis of cypermethrin enrichment cultures and bioremediation of cypermethrin contaminated soils. *Journal of Basic Microbiology*, vol. 55, no. 7, pp. 819-829. http://dx.doi.org/10.1002/jobm.201400805. PMid:25656248.

ALI, F., SHAKEELA, Q., UZMA, B., BIBI, A., NAJEEB, B., UR RAHMAN, A., SHAH, M. and AHMED, S., 2022. Antimicrobial resistance pattern and phenotypic detection of Extended spectrum beta lactamase-and Metallo beta lactamase-producing *Pseudomonas aeruginosa* isolated from indoor-patients suffering ear discharge. *Kuwait Journal of Science*, vol. 49, pp. 1-9. http://dx.doi.org/10.48129/kjs.10773.

ARMSTRONG, A. and PHILLIPS, K., 1998. *Controlling environmentat damage from sheep DIP chemicals*. UK: Environment Agency.

BARGHOUTHI, S.A., 2011. A universal method for the identification of bacteria based on general PCR primers. *Indian Journal of Microbiology*, vol. 51, no. 4, pp. 430-444. http://dx.doi.org/10.1007/s12088-011-0122-5. PMid:23024404.

BHATT, P., BHATT, K., SHARMA, A., ZHANG, W., MISHRA, S. and CHEN, S., 2021. Biotechnological basis of microbial consortia for the removal of pesticides from the environment. *Critical Reviews in Biotechnology*, vol. 41, no. 3, pp. 317-338. http://dx.doi.org/10.1080/07388551.2020.1853032. PMid:33730938.

BHATT, P., HUANG, Y., ZHAN, H. and CHEN, S., 2019. Insight into microbial applications for the biodegradation of pyrethroid insecticides. *Frontiers in Microbiology*, vol. 10, pp. 1778. http://dx.doi.org/10.3389/fmicb.2019.01778. PMid:31428072.

CHEN, S., LUO, J., HU, M., GENG, P. and ZHANG, Y., 2012. Microbial detoxification of bifenthrin by a novel yeast and its potential for contaminated soils treatment. *PLoS One*, vol. 7, no. 2, pp. e30862. http://dx.doi.org/10.1371/journal.pone.0030862. PMid:22348025.

CYCOŃ, M. and PIOTROWSKA-SEGET, Z., 2016. Pyrethroid-degrading microorganisms and their potential for the bioremediation of contaminated soils: a review. *Frontiers in Microbiology*, vol. 7, pp. 1463. http://dx.doi.org/10.3389/fmicb.2016.01463. PMid:27695449.

CYCOŃ, M., PIOTROWSKA-SEGET, Z., KACZYŃSKA, A. and KOZDRÓJ, J., 2006. Microbiological characteristics of a sandy loam soil exposed to tebuconazole and λ-cyhalothrin under laboratory conditions.

Ecotoxicology (London, England), vol. 15, no. 8, pp. 639-646. http://dx.doi.org/10.1007/s10646-006-0099-8. PMid:17033931.

DECOURTYE, A., DEVILLERS, J., GENECQUE, E., LE MENACH, K., BUDZINSKI, H., CLUZEAU, S. and PHAM-DELEGUE, M., 2005. Comparative sublethal toxicity of nine pesticides on olfactory learning performances of the honeybee *Apis mellifera*. *Archives of Environmental Contamination and Toxicology*, vol. 48, no. 2, pp. 242-250. http://dx.doi.org/10.1007/s00244-003-0262-7. PMid:15750780.

FAN, X., LIU, X., HUANG, R. and LIU, Y., 2012. Identification and characterization of a novel thermostable pyrethroid-hydrolyzing enzyme isolated through metagenomic approach. *Microbial Cell Factories*, vol. 11, no. 1, pp. 33. http://dx.doi.org/10.1186/1475-2859-11-33. PMid:22409882.

GONG, T., XU, X., DANG, Y., KONG, A., WU, Y., LIANG, P., WANG, S., YU, H., XU, P. and YANG, C., 2018. An engineered *Pseudomonas putida* can simultaneously degrade organophosphates, pyrethroids and carbamates. *The Science of the Total Environment*, vol. 628-629, pp. 1258-1265. http://dx.doi.org/10.1016/j.scitotenv.2018.02.143. PMid:30045547.

GRANT, R., DANIELL, T. and BETTS, W., 2002. Isolation and identification of synthetic pyrethroid-degrading bacteria. *Journal of Applied Microbiology*, vol. 92, no. 3, pp. 534-540. http://dx.doi.org/10.1046/j.1365-2672.2002.01558.x. PMid:11872130.

HINTZEN, E.P., LYDY, M.J. and BELDEN, J.B., 2009. Occurrence and potential toxicity of pyrethroids and other insecticides in bed sediments of urban streams in central Texas. *Environmental Pollution*, vol. 157, no. 1, pp. 110-116. http://dx.doi.org/10.1016/j.envpol.2008.07.023. PMid:18799250.

HOLT, J., KRIEG, N., SNEATH, P. and STALEY, J., 1994. *Bergey's manual of determinative bacteriology*. 9th ed. Baltimore: Williams and Wilkins.

IRAM, S., AHMAD, I., AHAD, K., MUHAMMAD, A. and ANJUM, S., 2009. Analysis of pesticides residues of Rawal and Simly lakes. *Pakistan Journal of Botany*, vol. 41, pp. 1981-1987.

JIN, Z., GUO, Q., ZHANG, Z. and YAN, T., 2014. Biodegradation of type II pyrethroids and major degraded products by a newly isolated Acinetobacter sp. strain JN8. *Canadian Journal of Microbiology*, vol. 60, no. 8, pp. 541-545. http://dx.doi.org/10.1139/cjm-2014-0104. PMid:25083550.

JUNG, J., BAEK, J.-H. and PARK, W., 2010. Complete genome sequence of the diesel-degrading *Acinetobacter* sp. strain DR1. *Journal of Bacteriology*, vol. 192, no. 18, pp. 4794-4795. http://dx.doi.org/10.1128/JB.00722-10. PMid:20639327.

LASKOWSKI, D.A., 2002. Physical and chemical properties of pyrethroids. In: G.W. WARE, ed. *Reviews of environmental contamination and toxicology*. New York: Springer.

MARTELLI, G. and GIACOMINI, D., 2018. Antibacterial and antioxidant activities for natural and synthetic dual-active compounds. *European Journal of Medicinal Chemistry*, vol. 158, pp. 91-105. http://dx.doi.org/10.1016/j.ejmech.2018.09.009. PMid:30205261.

OROS, D.R. and WERNER, I., 2005. *Pyrethroid insecticides: an analysis of use patterns, distributions, potential toxicity and fate in the Sacramento-San Joaquin Delta and Central Valley*. Richmond: San Francisco Estuary Institute Oakland.

PAUL, D. and MANDAL, S.M., 2019. Microbial adaptation and resistance to pesticides. In: S.M. MANDAL and D. PAUL, eds. *Bacterial adaptation to co-resistance*. Singapore: Springer.

PEARCE, D. W. and WARFORD, J. J., 2012. *Pakistan-Punjab private sector groundwater development project*. Washington, D.C.: World Bank Group.

RYALL, B., EYDALLIN, G. and FERENCI, T., 2012. Culture history and population heterogeneity as determinants of bacterial adaptation: the adaptomics of a single environmental transition. *Microbiology and Molecular Biology Reviews*, vol. 76, no. 3, pp. 597-625. http://dx.doi.org/10.1128/MMBR.05028-11. PMid:22933562.

SABIR, M.S., SHAHZADI, F., ALI, F., SHAKEELA, Q., NIAZ, Z. and AHMED, S., 2021. Comparative effect of fertilization practices on soil microbial diversity and activity: an overview. *Current Microbiology*, vol. 78, no. 10, pp. 3644. http://dx.doi.org/10.1007/s00284-021-02634-2. PMid:34480627.

SAKAMOTO, M., SUZUKI, N. and BENNO, Y., 2010. hsp60 and 16S rRNA gene sequence relationships among species of the genus Bacteroides with the finding that Bacteroides suis and Bacteroides tectus are heterotypic synonyms of Bacteroides pyogenes. *International Journal of Systematic and Evolutionary Microbiology*, vol. 60, no. Pt 12, pp. 2984-2990. http://dx.doi.org/10.1099/ijs.0.021154-0. PMid:20118288.

SHAKEELA, Q., LIAQAT, S., ALI, F., NIAZ, Z., HAYAT, A. and AHMED, S., 2020. Molecular identification of Quorum sensing and quorum quenching bacteria isolated from soil and hospital setup. *Preprints*. In press.

SINGH, B.K. and WALKER, A., 2006. Microbial degradation of organophosphorus compounds. *FEMS Microbiology Reviews*, vol. 30, no. 3, pp. 428-471. http://dx.doi.org/10.1111/j.1574-6976.2006.00018.x. PMid:16594965.

SINGH, P., SURI, C. and CAMEOTRA, S.S., 2004. Isolation of a member of *Acinetobacter* species involved in atrazine degradation. *Biochemical and Biophysical Research Communications*, vol. 317, no. 3, pp. 697-702. http://dx.doi.org/10.1016/j.bbrc.2004.03.112. PMid:15081396.

SONG, H., ZHOU, Z., LIU, Y., DENG, S. and XU, H., 2015. Kinetics and mechanism of fenpropathrin biodegradation by a newly isolated *Pseudomonas aeruginosa* sp. strain JQ-41. *Current Microbiology*, vol. 71, no. 3, pp. 326-332. http://dx.doi.org/10.1007/s00284-015-0852-4. PMid:26068594.

WANG, B.-Z., GUO, P., HANG, B.-J., LI, L., HE, J. and LI, S.-P., 2009. Cloning of a novel pyrethroid-hydrolyzing carboxylesterase gene from *Sphingobium* sp. strain JZ-1 and characterization of the gene product. *Applied and Environmental Microbiology*, vol. 75, no. 17, pp. 5496-5500. http://dx.doi.org/10.1128/AEM.01298-09. PMid:19581484.

WASI, S., TABREZ, S. and AHMAD, M., 2013. Use of *Pseudomonas* spp. for the bioremediation of environmental pollutants: a review. *Environmental Monitoring and Assessment*, vol. 185, no. 10, pp. 8147-8155. http://dx.doi.org/10.1007/s10661-013-3163-x. PMid:23519843.

WENDT-RASCH, L., FRIBERG-JENSEN, U., WOIN, P. and CHRISTOFFERSEN, K., 2003. Effects of the pyrethroid insecticide cypermethrin on a freshwater community studied under field conditions. II. Direct and indirect effects on the species composition. *Aquatic Toxicology*, vol. 63, no. 4, pp. 373-389. http://dx.doi.org/10.1016/S0166-445X(02)00202-3. PMid:12758003.

WESTON, D., HOLMES, R. and LYDY, M., 2009. Residential runoff as a source of pyrethroid pesticides to urban creeks. *Environmental Pollution*, vol. 157, no. 1, pp. 287-294. http://dx.doi.org/10.1016/j.envpol.2008.06.037. PMid:18676072.

WILSON, K., 2001. Preparation of genomic DNA from bacteria. *Current Protocols in Molecular Biology*, vol. 56, no. 1, pp. 24.1-24.5. http://dx.doi.org/10.1002/0471142727.mb0204s56. PMid:18265184.

WORLD HEALTH ORGANIZATION – WHO, 2009. *The WHO recommended classification of pesticides by hazard and guidelinesto classification*. Geneva: WHO.

ZHANG, C., JIA, L., WANG, S., QU, J., LI, K., XU, L., SHI, Y. and YAN, Y., 2010. Biodegradation of beta-cypermethrin by two *Serratia* spp. with different cell surface hydrophobicity. *Bioresource Technology*, vol. 101, no. 10, pp. 3423-3429. http://dx.doi.org/10.1016/j.biortech.2009.12.083. PMid:20116237.